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MODIFICATION OF DEVELOPMENT ON THE BASIS OF DIFFERENTIAL SUSCEPTIBILITY TO RADIATION.

II. Arbacia and Visible Light following Sensitization.

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Previous experiments by Bovie and Barr, '24, and by Hinrichs, '24, have clearly demonstrated that living organisms are differentially susceptible to lethal doses of radiation as well as to lethal doses of other physical and chemical agents (Child, '24). Those regions of the body which are physiologically the most active, are the first to die and disintegrate. There is a progressive antero-posterior disintegration gradient, coincident in lower animals and embryonic stages, with the main body axis. Disintegration gradients, as demonstrated by the method of photolysis, are essentially like those produced by other chemical and other physical agents.

With sublethal doses of radiation, as with sublethal doses of other agents, it is possible to modify embryonic development (Child, '24*b*, and Hinrichs, '24, and '25, now in press). With the proper intensity and duration of exposure, the developmental rates of various parts of the embryo may be relatively altered. Here again the development of those parts which are physiologically the most active, is most easily inhibited or entirely suppressed when doses are used which produce a permanent inhibition, and do not permit recovery or acclimation. On the other hand, if the inhibitory effect is slight or transitory, these same regions of high physiological activity will be the first to

acclimate or recover. (See Child, '24*b*, p. 247, for review of literature.)

Differential inhibition and differential recovery have been brought about (1) through the use of visible radiation (diffuse daylight, direct sunlight filtered through a glass dish containing water an inch deep, artificial light from two 1,500-watt photographic bulbs, and from the white-flame carbon arc) *plus* the action of the following sensitizing dyes: eosin, neutral red, benzoflavine, and methylene blue; and (2) by means of radiation from a mercury-vapor arc. (See also Child, '24*b*, footnote, p. 109.) Differential acclimation has been brought about by continuous exposure of sensitized eggs to diffuse daylight.

The development of eggs of Echinoderms has been repeatedly modified experimentally,[1] but the fact that there are relative differences in degree of susceptibility to modification of the various regions along the embryonic axes has not been generally noted. (For a more complete discussion of the results obtained by differentially modifying development, see Child, '16 and '23.) The main conclusions reached by this author regarding the forms attained in this way may be summed up as follows:

1. "In cases of differential inhibition the chief changes are decrease in size of oral lobe, which represents the apical region, decrease to zero in the angle of divergence between arms, approach of lateral parts toward the median line and in more extreme degrees fusion in the median line, the progressive obliteration of antero-posterior and medio-lateral differences and finally of apico-basal differences.

2. "The changes in differential acclimation and differential recovery are in the opposite direction. They consist in increase in size and over-development of the oral lobe, increase to 180° in the angle of divergence between the arms, and over-development of anterior and median as compared with posterior and lateral regions.

[1] For literature on modification of development see the following;

(*A*) Visible radiation following sensitization—Cooke and Loeb, '08, '09, Shippen, '07, and Loeb, '07, *a*, *b*.

(*B*) Ultraviolet radiation—Baldwin, '15, Stevens, '09, Ruppert, '24, Child, '24*b*, footnote, p. 109, Clarke, '22.

(*C*) X-rays and radium—Loeb, '22, Colwell and Russ, '24, Hinrichs, '25 (unpublished).

3. "Where the effect of differential inhibition persists after general recovery, a great over-development of the skeleton may occur, since the mesenchyme cells are relatively less inhibited than most, if not all other parts."

Larvæ whose development has been modified by means of radiation, present exactly the same picture as those produced by other means.

Method.—In general, in experiments in which differential inhibition has been produced, the method has been as follows: The eggs from a single female were strained through a cheese-cloth and washed. They were then stained in weak sea-water solutions of one or the other of the above dyes, for from ten to thirty minutes, again washed and then exposed for varying periods of time to radiation from one of the above-mentioned sources of light. (In some cases the eggs were exposed in a thin layer of a weak concentration of the dye.) Eggs were then washed and returned to sea-water and allowed to develop in the dark. The experimental controls consisted of (*A*) stained and unstained eggs (unexposed to strong light) which were allowed to develop in diffuse daylight, and another similar lot which developed in the dark, and (*B*) unstained eggs (exposed to strong light) and allowed to develop in the dark. Temperature was controlled by the addition of ice to the water bath surrounding the eggs.

Differential recovery followed in those cases where the inhibition was only transitory. Differential acclimation was obtained by allowing fertilized eggs to develop in sea-water solutions of dyes exposed to diffuse daylight.

Results.—Results of experiments made during the summers of 1923 and 1924 may be tabulated as follows:

A. Differential inhibition.....no recovery.
B. Differential inhibition.....differential recovery.
C. Differential inhibition.....general recovery.
D. Differential acclimation.

A. Differential Inhibition.—Since it is true that those regions which are relatively the most active physiologically, during a particular stage of growth, are also the most highly susceptible to the destructive action of lethal doses of chemical agents

(Child, '16, '20, '23), and since these regions are also the first to be modified by sublethal doses of such agents (Child, '16*b*, '24), it seemed likely that those regions of the developing embryo which showed the greatest degree of developmental modification (see also Child, '16*b*), as for example, the oral lobe and aboral arms, would also show the earliest evidences of disintegration in lethal doses of visible light following sensitization (Figs. 1–3).

Modification of development in these regions is brought about by an exposure made much earlier than at the pluteus stage. A short exposure made immediately after fertilization will produce abnormal inhibited development of these active regions. Two possibilities suggest themselves; either a general metabolic lowering of developmental rate at the time of exposure, produces an effect at first latent, and not appearing until later, at a highly susceptible stage of development; or, there is an early difference in the relative susceptibility of regions or "Anlagen" present in the fertilized egg. The latter seems more probable. (See also Hinrichs, '25, now in press.)

As a result of inhibition of development by means of radiation, forms are produced in which the anterior region of the developing embryo is relatively less developed than other regions. The oral lobe, which represents the apical region, and the aboral arms which together with the oral lobe, forms the anterior region of the pluteus, show the greatest effect (Figs. 7–11). The angle between the arms is greatly reduced. Often there is but a single median arm (Figs. 12, 13) or one partially fused anteriorly (Fig. 14).

If the inhibitory effect has been even more marked, the aboral arms and oral lobe may be undeveloped, and there remains an ovoid mass which may have short parallel skeletal arms embedded in the tissue, one on either side of the archenteron, which latter has but one opening to the exterior (Fig. 15). A final stage of inhibition shows an apolar spherical mass with non-directive swimming (Fig. 16). Exogastrulæ (Figs. 17–22) are also commonly found in cultures in which inhibition has been produced. (See also Herbst, '95, and MacArthur, '24.)

B. Differential Recovery.—As examples of differential recovery obtained by short early exposures in which the harmful effect is

transitory, wide-angled forms, some up to 180°, with over-developed oral lobes give evidence that the median anterior region has recovered from a slight inhibition and that its rate of growth has surpassed that of other regions (Figs. 23–27).

C. General Recovery.—Individual differences in eggs from the same female (see also Medes, '17, and Tennent, '10) produce differences in degree, and even in direction of effect; but the larger number (90 per cent. or more) of the embryos in a given lot may, for example, show differential inhibition, while the rest of the embryos in the lot may show general recovery or no effect at all. The degree of effect produced is roughly proportional to the dosage (see Figs. 58, 63) and is also greater at higher temperatures. (See Bovie and Daland, '23, Bovie, '18.)

D. Differential Acclimation.—Differential acclimation results when the inhibitory effect of radiation is mild enough to permit adjustment, for example, when the eggs are exposed continuously, in weak concentrations of dyes, to diffuse daylight, or are kept in stronger concentrations for several days in the dark. The resulting forms, are wide-angled and have over-developed oral lobes like those appearing after differential recovery. (See Figs. 37–39.)

In higher concentrations there is a differential effect on development even in the dark (Figs. 33–39); but in order that the lower concentrations may be effective, there must have been at some time, preferably during early development, an exposure of sufficient duration to radiation of the proper intensity. The effects produced under these conditions can to some extent be controlled.

Frequently there appears a larger number of aboral arms than normal (Figs. 40–41). Probably these result from the activity of mesenchyme cells which under normal conditions are inhibited in development. (Child, '16*a*.) (The possibility of stimulation of calcium metabolism by radiation should also be considered.)

Long exposures to radiation (7 minutes under Tungsten bulb, or 90 minutes in sunlight) produce some differential inhibition in unstained eggs (Figs. 42–47). In our previous work with activation of Echinoderm eggs by photodynamic action it was found that strong illumination frequently induces membrane formation, especially in eggs having more than the usual quantity of pigment, echinochrome. (Lillie and Hinrichs, '23.)

Two-day larvæ show greater effects of radiation than do eight-day larvæ, in most cases, indicating some degree of slow regulation and general recovery.

E. Experiments with Sperm.—Much work has been done in producing developmental modifications by subjecting one or the other germ cell to the deleterious action of chemicals (mostly anesthetics) and physical agents (particularly X-ray and radium) before fertilization. More work along these lines is contemplated with visible radiation following sensitization and a discussion of the literature will be reserved for a later paper.

It seems interesting to note, however, in connection with the work on differential developmental modifications, that eggs fertilized by sperm treated photodynamically (see also Bohn and Drzewina, '23 and Hertwig, '20), and eggs treated photodynamically before fertilization with normal sperm, develop into larvæ showing typical differential effects (Figs. 49–63).

Long exposures of sperm produce differential inhibition in the fertilized egg (Figs. 49–53, 55, 56), while shorter exposures produce a less harmful effect on the sperm, the resulting embryo shows indications of differential acclimation (Figs. 58–63).[1]

Discussion.—Concerning the action of a sensitizing dye like eosin, it is interesting to recall some of the conclusions reached by other experimenters,[2] namely; 1. The fundamental observations of von Tappeiner, '09, Hertel, '05, and Leo Loeb and his students, '07*a*, *b*, and the more recent evidence of Metzner, '21, '23, Schanz, '18, '21, Lillie and Hinrichs, '23, Hinrichs, '24, and Pereira, '25, have conclusively shown that eosin solutions, except in high concentrations depend for their toxicity on the presence of light, and that the resulting photodynamic action is probably primarily a surface effect (Lillie and Hinrichs, '23, and Pereira, '25).

2. Light to be effective must be absorbed (Grotthus-Draper Law), also see Hausmann, '23, p. 7. Ultraviolet radiation is directly absorbed by living protoplasm, primarily at the surface (Lillie and Baskervill, '22*a*, *b*). Visible radiation in the presence

[1] The outline sketches on Plates I. and II. were made with a Bausch and Lomb euscope at intervals during development, and represent a magnification of about 60 diameters.

[2] For a complete review, see Dubois, '14, Hausmann, '23, and Bayliss, '20.

of a sensitizer like eosin appears to act primarily at the surface, as for example in membrane elevation in *Asterias* eggs (Lillie and Hinrichs, '23).[1] This effect may later be transmitted to adjacent regions (Hinrichs, '24, Henri, '12, Schleip, '23).

3. The action of light is a differential one (Bovie, '24, Hinrichs, '24, '25). This is evident from the fact that disintegration in lethal doses of radiation in the two spectral regions tested, ultraviolet and the blue-green region of the visible, follows an anteroposterior gradient along the axis of the body of lower organisms or embryos.

4. On the basis of such differential susceptibility to radiation, it has been possible to modify the development of eggs of *Fundulus* by exposure to ultraviolet radiation (Hinrichs, '25, unpublished). In the resulting embryos, those regions of the body which have the highest rates of physiological activity are the first to be modified. Accordingly forms appear in which the central nervous system, the heart and circulatory system, or the developing tail region are abnormal. Such effects may be produced by exposures made during the first ten minutes after fertilization, indicating that at this early stage physiological differences are already present which determine differences in susceptibility, *i.e.*, such differences exist even before there is any morphological differentiation visible.

5. Modification of development in *Arbacia* eggs is likewise dependent on such early differences in susceptibility. Child ('16*a*) has been able to produce developmental modifications with chemicals, and the results with radiation (see Child, '24*b*, footnote, p. 109) give further evidence, (1), of the non-specific nature of such susceptibility differences, and (2), of the dependence of these differences on quantitative differences along the physiological axes. Obviously there can be nothing specific, for example, in the inhibition of development of the oral lobe region by two such widely different agents as KCN on the one hand, and ultraviolet radiation, or visible radiation following sensitization on the other.

A region of the body which has a relatively high rate of physio-

[1] Loeb, '07, Shippen, '07, and Pereira, '25, have found that eosin stains only dead cells. In other words, destruction of the surface film precedes cell cytolysis and subsequent staining by eosin.

logical activity, as for example, the oral lobe region, is very intimately dependent on its environment for the continuance of its life processes. The more active such processes are, the more quickly will such a region register an incompatibility in its relation to its environment. Consequently, large doses of chemical or physical agents will produce a gradient of death and disintegration progressing from regions of high metabolic rate to those of lower rate; while in the case of sublethal doses of these agents, the result is an interference (temporary or permanent), with the normal growth and developmental capacities of the same region, the degree of interference being in general proportional to the intimacy of dependence of such regions on the environment.

As stated above, experiments in which normal eggs were fertilized by sperm which had been photodynamically treated, gave results of a similar kind. Since the embryo develops from a zygote, the sperm component of which is responsible for the bringing in of a protoplasmic system which is toxic for normal development of the egg protoplasm, and which remains a part of the developing embryo, there is obviously no result possible other than inhibition or acclimation. And since the developing embryo becomes differentially modified, it appears that the egg at the time of fertilization by the injured sperm, already shows evidence of the difference in susceptibility of its various parts.

Whatever the direct effect of light on living protoplasm, whether it be electron emission (Clark, '22) or heat sensitization (Bovie, '13, '23), there is certainly a fundamental relationship between the effectiveness of its action and the degree of physiological activity of the protoplasm acted upon. In general, the degree of physiological activity and the rate of oxygen consumption parallel each other, and since light action requires oxygen for its maintenance (under conditions of the experiment), it seems likely that there is some connection between the degree of effectiveness of light action and the rate at which the tissue consumes oxygen. (Further experimental data are necessary for the affirmation of such a premise.)

Of the dyes used, eosin and benzoflavine are adsorbed by the cell surface while neutral red and methylene blue are intra vitam dyes. Aside from their photodynamic properties, the latter two

have shown themselves to be more toxic in comparable concentrations than eosin and benzoflavine even in the dark. (See also MacArthur, '24.) That they also possess photodynamic properties is evident from the fact that their toxicity, in weak concentrations, is augmented in the presence of light. (See Cooke and Loeb, '08, Bohn and Drzewina, '23, O. Hertwig, '12.) The present experiments have shown that methylene blue in comparable concentrations is about thirty times as toxic as eosin. An exposure of approximately fifteen times the duration is required to produce the same degree of inhibition with eosin as with one half the concentration of methylene blue. This is to be related to the position of the absorption band of methylene blue, which is near the region of maximum emission in the energy spectrum.

Eosin and benzoflavine have long been known as photodynamic sensitizers. Benzoflavine was found by Viale, '19, to be only slightly effective. On the other hand, eosin has been most commonly used as a sensitizer.[1] Eosin absorbs in the blue-green region, neutral red in the blue and blue-green, methylene blue in the orange and red, and benzoflavine in the azure-violet of the visible spectrum. Sensitization with these dyes makes tissues sensitive to the action of rays of a wave-length corresponding to the absorption band of the particular sensitizer used.

In comparing the relative effectiveness of the various light sources, it may be noted that with 1/10,000 eosin (30 minutes' staining), exposure to radiation from the photographic bulb was 90/7 times as effective in producing 100 per cent. inhibition as exposure to sunlight. This estimate is based on a ratio of the periods required in each case to produce inhibition in all eggs. Temperature was controlled.

Conclusions.—These experiments, in which the embryonic development of Arbacia was modified by means of visible radiation following sensitization, offer further evidence for the following:

1. The effectiveness of photodynamic sensitization depends upon (*A*) exposure of the sensitized system (in this case, fertilized eggs) to radiation of sufficient intensity and duration (dosage),

[1] See Ledoux-Lebard, '02, Hertel, '05, von Tappeiner, '07, Loeb, '07*a*, *b*, Cooke and Loeb, '08, '09, O. Hertwig, '12, Schanz, '18, Clark, '22, Metzner, '21, '23, Lillie and Hinrichs, '23, Viale, '21, '23, '24, Hinrichs, '24, and Pereira, '25.

which in order that it may be absorbed and so become effective, must have, (*B*) a wave-length range including that of the absorption band of the particular sensitizer used.

2. The susceptibility to visible radiation following sensitization with the following sensitizing dyes (eosin, benzoflavine, neutral red, and methylene blue) is differential, *i.e.*, regions of high physiological activity are first to be modified in their development. Differential inhibition, recovery, and acclimation have been produced in this way.

3. It is possible to produce these effects (as with *Fundulus* and ultraviolet radiation) by exposure during the first few minutes after fertilization, indicating an early difference in susceptibility of various regions of the egg. Differential modification is also obtained by fertilizing normal eggs with photodynamically treated sperm.

4. These experiments confirm the view of the nonspecific and quantitative nature of susceptibility relations along the body axis.

I wish to thank Dr. Ralph Lillie of the Dept. of Physiology for his coöperation in the working out of this problem. Acknowledgment is also due to Dr. C. M. Child of the Dept. of Zoölogy, and to Dr. S. E. Pond of the Dept. of Physiology, Washington University Medical School, for helpful suggestions.

LITERATURE CITED.

Baldwin, W. M.

'15 The Action of Ultraviolet upon the Frog's Egg. Anat. Rec., Vol. 9, pp. 365–381.

Bayliss, W. M.

'20 Principles of General Physiology. London.

Bohn, G., and Drzewina, A.

'23 Action toxique du rouge neutre en presence de la lumière. C. rend. Soc. Biol., Vol. 89, No. 24, pp. 386–388.

Bovie, W. T.

'13 Sensitization of Albumin and *Paramœcium* to Heat. Science, Vol. 37, pp. 373–375.

Bovie, W. T., and Klein, A.

'18 Sensitization to Heat Due to Exposure to Light of Short Wave-lengths. Jour. Gen. Physiol., Vol. 1, pp. 331–336.

Bovie, W. T., and Barr, C. E.

'24 Photocytolysis as a Measure of Metabolic Activity. Science, Vol. 59, No. 1514, p. 22.

Bovie, W. T., and Daland, G. A.

'23 New Experiments—Sensitization to Heat Due to Exposure to Light of Short Wave-length. Am. Jour. Physiol., Vol. 66, pp. 55–66.

Child, C. M.

'16 Experimental Control and Modification of Development in the Sea Urchin in Relation to Axial Gradients. Jour. Morph., Vol. 28, pp. 65–133.

'16*b* Axial Susceptibility Gradients in the Early Development of the Sea Urchin. BIOL. BULL., Vol. 30, pp. 391–405.

'20 Some Considerations Concerning the Nature and Origin of Physiological Gradients. BIOL. BULL., Vol. 39, pp. 147–187.

'23 The General Relation between Susceptibility and Physiological Condition. Arch. Int. Med., Vol. 32, pp. 647–662.

'24 Modification of Development in Relation to Differential Susceptibility. Am. Nat., Vol. 58, pp. 237–253.

'24*b* Physiological Foundations of Animal Behavior. U. of C. Press.

Clark, J. H.

'22 The Physiological Action of Light. Physiol. Rev. Vol. 2, No. 2, pp. 277–309.

Colwell, H. A., and Russ, S.

'24 Radium, X-rays, and the Living Cell. London.

Cooke, E., and Loeb, L.

'08 Comparative Toxicity of NaCl and Staining Solutions on the Embryo of *Fundulus*. Proc. Soc. Exp. Biol. and Med., Vol. 6, pp. 113–115.

'09 Über die Giftigkeit einiger Farbestoffe fur die Eier von Asterias und von *Fundulus*. Bioch. Zeit., Vol. 20, No. 1, pp. 167–177.

Dubois, R.

'14 La Vie et la Lumiere. Paris.

Hausmann, W.

'23 Grundzüge der Lichtbiologie und Lichtpathologie. Berlin.

Henri, V.

'12 Comparison de l'action des rayons U.V. sur les organismes avec les reactions photochimiques simples et complexes. C. rend. Seances, Vol. 73, pp. 659–661.

Herbst, C.

'95 Experimentelle Untersuchungen, Mitth. d. Zool. Stat. zu Neapel, Vol. 11, pp. 136.

Hertel, E.

'05 Einwirkung von Lichtstrahlen auf den Zellteilungsprozess. Zeit. f. Allg. Physiol., Vol. 5, pp. 95–122.

Hertwig, O.

'12 Veränderung der idioplasmatischen Beschaffenheit der Samenfäden durch physikalische und chemische Begriffe. Sitzungsber. d. Königl. Preuss. Akad. d. Wiss, Vol. 31, pp. 553–571.

Hinrichs, M. A.

'24 A Demonstration of the Axial Gradient by Means of Photolysis. Jour. Exp. Zoöl., Vol. 41, pp. 21–31.

'25 Modification of Development on the Basis of Differential Susceptibility to Radiation. 1. *Fundulus* and Ultraviolet Radiation. Jour. Morph. and Physiol. (unpubl.).

Lillie, R. S., and Baskervill, M.

'22 The Action of Ultraviolet Rays on Starfish Eggs. Am. Jour. Physiol., Vol. 61, No. 1, pp. 57–71.

'22*b* The Action of Ultraviolet Rays on *Arbacia* Eggs Especially as Affecting the Response to Hypertonic Sea-water. Am. Jour. Physiol., Vol. 61, No. 2, pp. 272–288.

Lillie, R. S., and Hinrichs, M. A.

'23 Activation of Starfish and Sea-urchin Eggs by Photodynamic Action. (Abstract.) Anat. Rec., Vol. 26, p. 370.

Ledoux-Lebard.

'02 Action de la lumière sur la toxicité de l'eosine et de quelques autres substances. Annals de l'Inst. Pasteur., Vol. 16, pp. 587–594.

Loeb, L.

'07 Über den Einfluss des Lichtes auf die Färbung und Entwickelung von Eiern von Asterias in Lösungen verschiedener Farbstoffe. Arch. f. Entw., Vol. 23, pp. 359–378.

'07*b* The Effect of Light on the Staining of Cells. Proc. Soc. Exp. Biol. and Med., Vol. 4, pp. 94–96.

'22 Effects of Roentgen Rays and Radioactive Substances on Living Cells and Tissues. Jour. Cancer Res., Vol. 7, pp. 229–282.

MacArthur, J. W.

'24 Physiological Interpretation of Exogastrulation and Related Modifications in Echinoderm Embryos. BIOL. BULL., Vol. 46, No. 2, pp. 60–87.

Medes, G.

'17 A Study of the Causes and Extent of Variation in the Larvæ of *Arbacia punctulata*. Jour. Morph., Vol. 30, No. 1, pp. 317–432.

Metzner, P.

'21 Zur Kenntnis der photodynamischen Erscheinung; Die induzierte Phototaxis bei Paramœcium caudatum. Bioch. Zeit., Vol. 113, pp. 145–175.

'23 Induzierter Phototropismus an Keimwurzeln. Ber. d. Deut. Bot. Ges., Vol. 41, No. 6, pp. 268–274.

Pereira, J. R.

'25 On the Combined Toxic Action of Light and Eosin. Jour. Exp. Zoöl., Vol. 42, No. 1, pp. 257–262.

Ruppert, W.

'24 Differences in Susceptibility of *Ascaris* Eggs in Various Stages of Development to the Effects of Ultraviolet Rays. Zeit. f. wissen Zool., Vol. 123, No. 1, pp. 103–155.

Schanz, F.

'18 Biochemische Wirkung des Lichtes. Pflüger's Arch., Vol. 170, pp. 646–676.

'21 Die physikalischen Vorgänge bei der optischen Sensibilisation. Pflüger's Arch., Vol. 190, pp. 311–320.

Shippen, L. P.

'07 The Effect of Light on the Development of Ova in Eosin Solutions. U. of Penn. Med. Bull., Vol. 19, pp. 316–319.

Schleip, W.

'23 Die Wirkung des ultravioletten Lichtes auf die morphologischen Bestandteile des Ascariseies. Arch. f. Zellf., Vol. 17, No. 3, pp. 289–367.

Stevens, N. M.

'09 The Effect of Ultraviolet Light on the Developing Eggs of *Ascaris meg.* Arch. f. Entw., Vol. 27, pp. 622–639.

von Tappeiner, H.

'09 Die photodynamische Erscheinung. (Sensibilisierung durch fluorescierende Stoffe.) Ergeb. d. Physiol., Vol. 8, pp. 698–741.

Tennent, D. H.

'10 Variation in Echinoid plutei. Jour. Exp. Zoöl., Vol. 9, No. 4, pp. 657–714.

Viale, G.

'19 Ricerche sui Fenomeni Fotodinamici. 1. Insorgenzie e Condizioni del Fenomeno Fotodinamico. Arch. di Scienze Biol., Vol. 1, No. 2, pp. 78–91.

'21 Ricerche, etc. III. Il Fenomeno Fotodinamico nel cuore isolato. Arch. di Scienze Biol., Vol. 2, No. 3, pp. 231–278.

'23 Ricerche, etc. IV. Raggi X e Fenomeni Fotodinamici. Arch. de Scienze Biol., Vol. 4, No. 3, pp. 323–331.

'24 Differenciation entre phenomens photochimiques et phenomens photodynamiques. Arch ital. de Biol., Vol. 73, No. 1, pp. 19–23.

Explanation of Plate I.

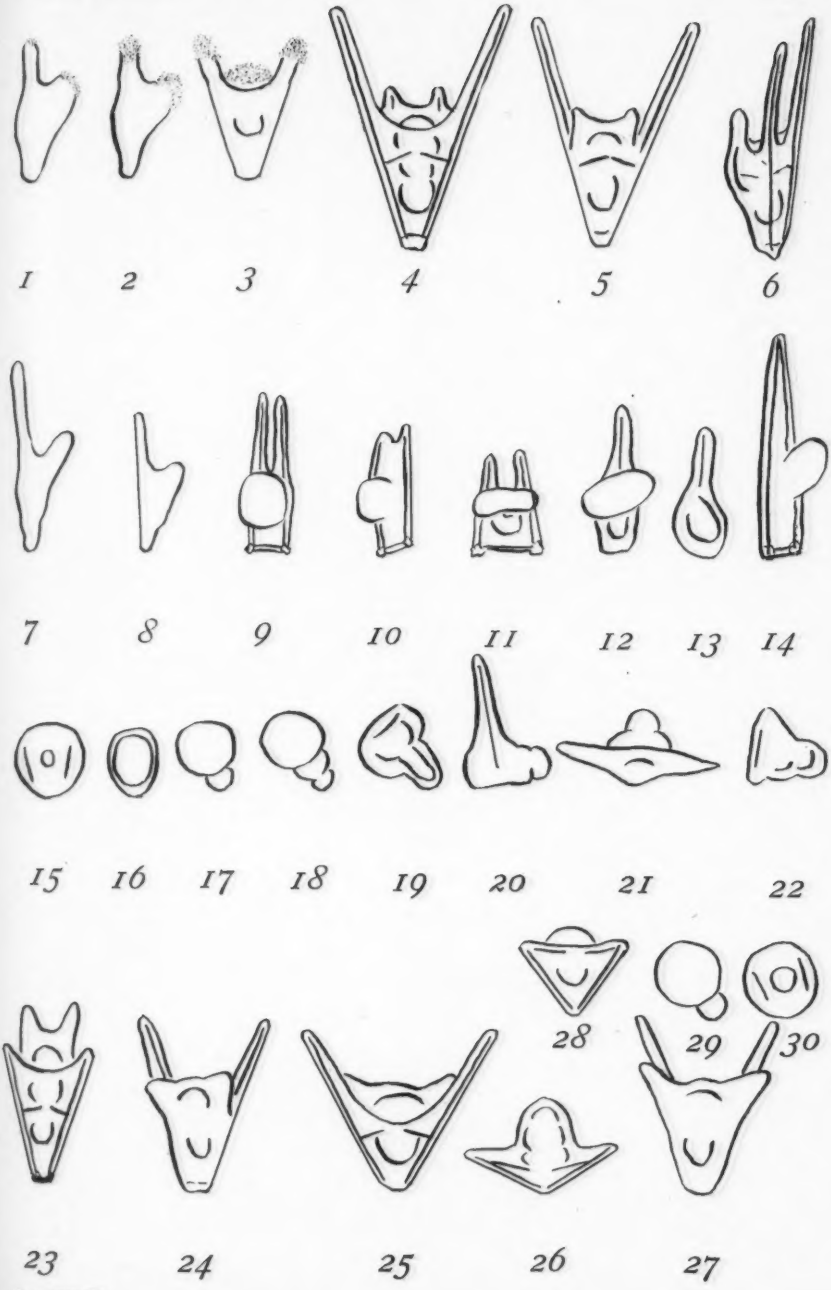
Figs. 1–3. Young plutei showing successive stages of early disintegration by photolysis. Lateral view, Fig. 2. Aboral view, Fig. 3.

Figs. 4–6. Normal three-day plutei. Fig. 4, Unstained, developed in dark. Fig. 5, Stained, developed in dark. Fig. 6, Lateral view of Fig. 5.

Figs. 7–22. Differential inhibition. Figs. 7–8, lateral view. Aboral arms and oral lobe slightly reduced. Figs. 9–11. Approximation of arms indicating inhibition of development of the median anterior region. Figs. 12–13, Complete reduction of median anterior region. Single median arm. (Figs. 7–13—Two-day larvæ. For normals see Fig. 48.) Fig. 14. Three-day larva. Union of two arms anteriorly, due to the reduction of the median anterior region. Figs. 15–16. More complete inhibition of development. Figs. 17–21. Exogastrulæ, indicating a difference in susceptibility to growth-inhibiting influences. Endoderm cells have continued to grow at the expense of ectoderm cells. Fig. 21. The widely diverging aboral arms indicate a secondary differential recovery of the median anterior region.

Figs. 23–27. Differential recovery. The anterior median region has recovered from a temporary inhibition and has regained and finally surpassed its normal growth rate. Oral lobe is over-developed. (Fig. 26. The angle of divergence between the aboral arms is much greater than normal.)

Figs. 28–30. Differences in degree of action of visible radiation. (*A*) Exposed without previous staining, Fig. 28. (*B*) After staining, Figs. 29, 30. The inhibitory effect is greater in *B*. Both *A* and *B* were exposed for the same period of time.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M. A. HINRICHS.

EXPLANATION OF PLATE II.

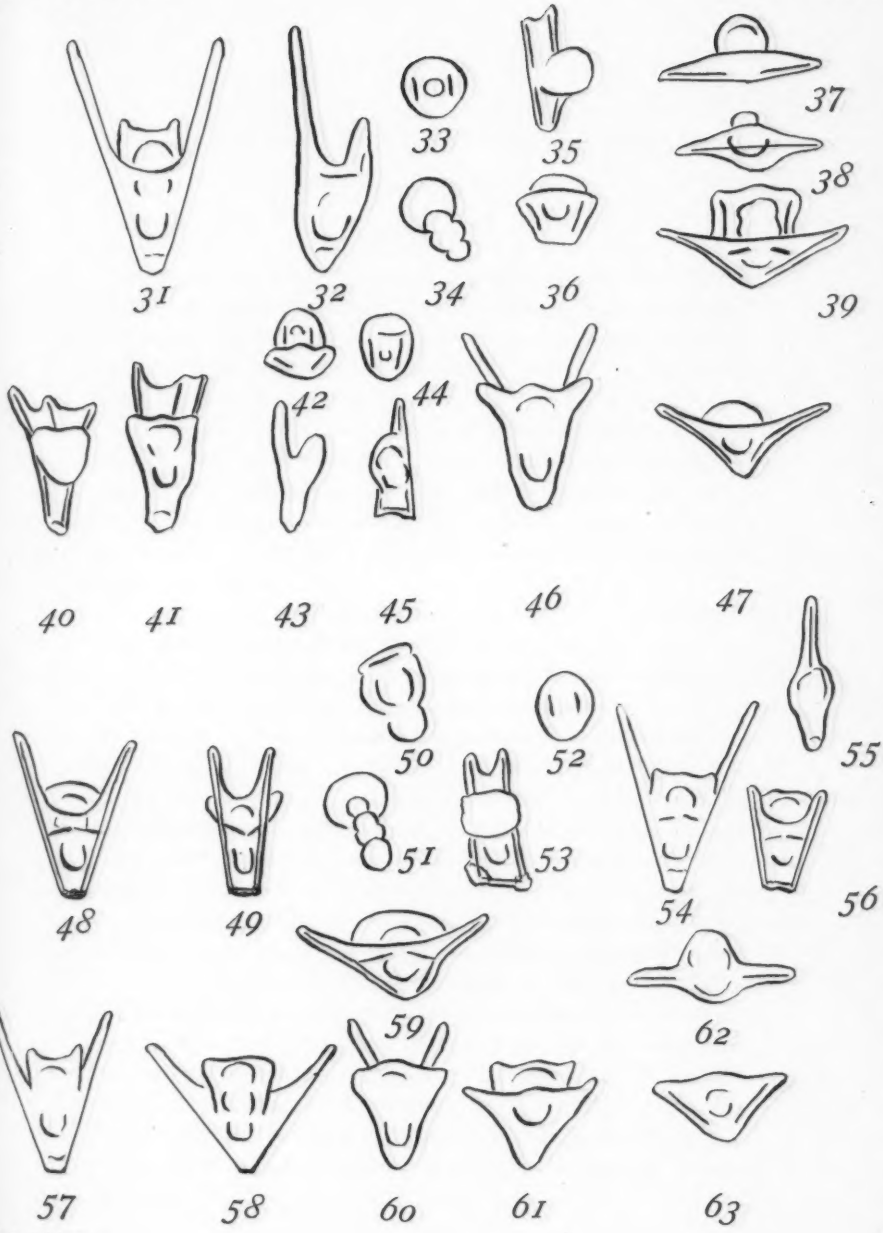
FIGS. 31–32. Normal two-day larvæ.

FIGS. 33–39. Effect of development in more concentrated solutions of sensitizing dyes in the dark. Figs. 33–36 show differential inhibition. Figs. 37–39 show differential acclimation. (Figs. 33, 34, 37, 38, development in eosin solution in the dark. Figs. 35, 39, benzoflavine, dark. Fig. 36, methylene blue, dark.)

FIGS. 40–41. Overproduction in the number of aboral arms. Fig. 40. Unstained sperm exposed to radiation from photographic bulb for 30 minutes × normal egg. Fig. 41. Stained sperm (stained in the dark for 30 minutes before fertilizing normal eggs.)

FIGS. 42–47. Effect of light action alone. (No previous sensitization.) Figs. 42–45 show differential inhibition. Figs. 46, 47 show differential recovery. (Figs. 42, 43, 44. Two-day larvæ exposed to direct sunlight for 90 minutes, immediately after fertilization. No previous staining. Fig. 45. Two-day larvæ. Exposed to radiation from photographic bulb for 7 minutes. No previous staining. Figs. 46, 47. Six-day larvæ. Exposed to direct sunlight for 90 minutes. No previous staining.)

FIGS. 48–63. Effect of fertilizing normal eggs with photodynamically treated sperm. Fig. 48. Normal two-day larva. Control. Fig. 49. Differential inhibition followed by slight differential acclimation. Normal eggs fertilized by eosin-stained sperm exposed to radiation from photographic bulb for one minute. Figs. 50, 51. Exogastrulæ produced as a result of fertilizing normal eggs with sperm, eosin-stained, and exposed as above for 4 minutes. Figs. 52, 53, 55, 56 show differential inhibition. (Fig. 55 is result of exposing eosin-stained sperm to diffuse daylight for 30 minutes. Figs. 52, 53, 56, are the result of a 10-minute exposure of sperm before fertilization.) Fig. 54. General recovery. Appears normal. Normal eggs fertilized with eosin-stained sperm which had been exposed to radiation from photographic bulb for 1 minute. Fig. 57. General recovery. Stained sperm had been exposed for 30 minutes to radiation from bulb. Figs. 58–63. Differential acclimation. Fig. 58. Stained sperm exposed for one minute. Radiation from bulb. Figs. 59, 60. Result of 4-minute exposure. Figs. 61–63. Ten-minute exposure.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M. A. HINRICHS.

FAUNA OF PENIKESE ISLAND, 1923.

(Edited by R. E. Coker.)

Introduction.

On the occasion of the fiftieth anniversary of the founding of the Agassiz laboratory on Penikese, a biological reconnaissance of the island was undertaken jointly by botanists and zoölogists from the Marine Biological Laboratory and the Fisheries Biological Station of the United States Bureau of Fisheries at Woods Hole, Mass. The studies of the flora have been published under the editorship of Professor I. F. Lewis in *Rhodora*, Vol. 26, Nos. 310, 311 and 312, 1924. The zoölogical collections and studies were made by the workers to be named hereafter, while the arrangement of the report for publication has been entrusted to the present editor.

With regard to the fauna, as well as to the flora, the collections were made by large parties, but chiefly on one day in each case. Practical considerations made it necessary for the zoölogists to restrict attention to the larger animals existing above the tide line, that is to say, to the non-microscopic animals living on the land or in the small freshwater and brackish ponds that are described by Miss Shaw in the report on Flora. Notwithstanding the number of collectors and the varied interests and experiences represented by them, the time spent on Penikese imposed an obvious limit to the exhaustiveness of the collections, especially since the fauna of the island is comprised largely in the group of Arthropods, so many of which are seasonal in appearance.

In the following list are the names of the persons comprising the zoölogical collecting expedition to Penikese Island, August 7, 1923, with the names of the respective institutions with which they were permanently engaged. The abbreviation "M.B.L." for Marine Biological Laboratory or "U.S.F.L." for United States Fisheries Laboratory, is used to denote the local laboratory to which each was temporarily attached during the summer of 1923.

F. E. Chidester, West Virginia University. U.S.F.L.
R. E. Coker, University of North Carolina. U.S.F.L.
Arch E. Cole, Northwestern University. M.B.L.
Manton Copeland, Bowdoin College. M.B.L.
J. A. Dawson, Dalhousie University. M.B.L.
S. R. Detwiler, Harvard University. M.B.L.
Samuel W. Geiser, Washington University. M.B.L.
Robert A. Goffin, U. S. Bureau of Fisheries.
Benjamin Grave, Wabash College. M.B.L.
Caswell Grave, Washington University. M.B.L.
George M. Gray, Marine Biological Laboratory.
Walter N. Hess, De Parmo University. M.B.L.
Hoyt S. Hopkins, Baylor Medical College. M.B.L.
Ondess L. Inman, Antioch College. M.B.L.
Ivey F. Lewis, University of Virginia. M.B.L.
Edwin Linton, Washington and Jefferson College and University of Georgia Medical Dept. U.S.F.L.
S. O. Mast, Johns Hopkins University. M.B.L.
C. E. McClung, University of Pennsylvania. M.B.L.
Everett C. Myers, Johns Hopkins University. M.B.L.
James T. Penney, University of North Carolina. U.S.F.L.
Albert M. Reese, West Virginia University. U.S.F.L.
A. H. Sturtevant, Carnegie Institute of Washington. M.B.L.
Henry C. Tracy, University of Kansas. U.S.F.L.
Arthur D. Whedon, North Dakota Agricultural College. M.B.L.
H. L. Wieman, University of Cincinnati. M.B.L.
Charles B. Wilson, Massachusetts State Normal School, Westfield. U.S.F.L.
John E. Wilson, Student Harvard Law School. U.S.F.L.
F. E. Wood, Illinois Wesleyan University. M.B.L.
Lorande Loss Woodruff, Yale University. M.B.L.
Donnell B. Young, Carleton College. M.B.L.

The following junior members of the expedition rendered helpful service in collecting:

Robert E. Coker, Jr.
Frederick C. Copeland.
Preston S. Copeland.

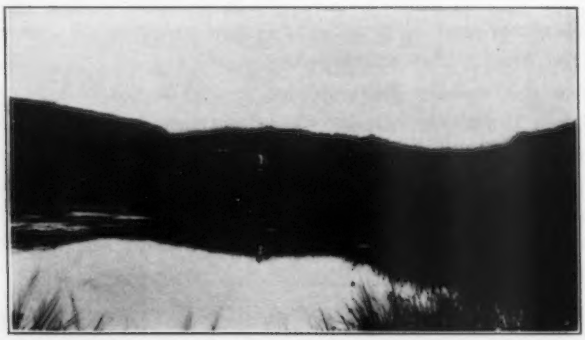
The party was conveyed from the Fisheries Laboratory at Woods Hole to Penikese Island by the U. S. Fisheries Steamer *Pharalope*. Collecting apparatus of every character as available was taken; there were seines, townets, dipnets, butterfly nets, sweeping nets, spade, rakes, gun, dipper, spoon, rat traps, mouse traps, etc., with necessary reagent for killing and preserving, and containers of various sorts, besides maps, labels, salinometers and cameras. The methods employed may be inferred from the apparatus taken.

It was originally contemplated to report the collections by station and habitat, but the character of the fauna of the island to which reference is made on page 25 below, made it evident that no particularly useful purpose would be served by adhering to a plan that would have both complicated the study and greatly extended the report.

Penikese Island. Northwest part, looking north from Pond 2-B.
(See footnote to p. 27.)

The study and identification of material was done in part by certain members of the collecting party, and in part by specialists associated with the United States National Museum and the Boston Society of Natural History; cordial acknowledgments are due to those institutions for the assistance that was so freely rendered. Appropriate credit to individuals is given in connection with the lists of species beginning on page 26, but in this place particular mention should be made of the following persons as the virtual authors of the systematic portion

of this report: Messrs. C. W. Johnson (insects of several orders), A. H. Sturtevant (Diptera), C. B. Wilson (Odonata, aquatic Hemiptera, and aquatic Coleoptera), C. E. McClung (Orthoptera), Manton Copeland (Lepidoptera—butterflies), J. H. Emerton (Arachnida), Ondess L. Inman and Everett C. Myers (Birds).



Pond 1-*D*. (See footnote to p. 27.)

The general character of the island and its ecological features as regards the vegetation have been described by Lewis and Miss Shaw in the report previously mentioned. While the vegetational environments are of interest in connection with the consideration of the fauna, it would be superfluous to restate here the data to be found in that publication, especially in the section on "Ecology of Penikese" by Miss Margaret F. Shaw. It is necessary to give only a single quotation from Lewis (below) and to say that the small rocky island is practically devoid of trees, and, except where denuded of all vegetation in some of the nesting grounds of terns, is given over to grasses and other small plants, with rarely a low growing shrub or willow.

"The island Penikese is a remnant of the terminal moraine now seen in the Elizabeth Islands. It is about two thirds of a mile long and half as broad, with a broadly spatulate point for convenience of reference called Tub Point, extending further to the east for another third of a mile. Its contour is dominated by low hills on the main body of the island, with depressions here and there which may be ponds of a rather temporary character (map).

"The original vegetation, like that of neighboring islands, is said by Jordan to have been of a forest type, with pitch pine red cedar, red maple, shagbark, shadbush, poplar birch, hornbeam, and two or three species of sumac. In 1873 there was 'no trace left save the rotten roots of a solitary beech stump and a few branches of red cedar and red maple (?) found buried in the muck of a small swamp.' Cutting of the original timber had been followed by the introduction of sheep, which remained on the island until about 1910. From 1905 to 1921 Penikese was used by the State of Massachusetts as the site of a leprosarium. After the removal of the lepers in 1921 the island has been uninhabited except for the caretaker and his wife.

Pond 2-*D*. (See footnote to p. 27.)

"Jordan says that his 'list may have a special interest to future students, and also a general interest to botanists, as showing which plants survive a prolonged struggle for existence against grass and sheep.' If the State retains possession of Penikese and establishes a bird sanctuary there, the continued history of the development and succession of plants and animals cannot fail to be of interest and importance. Surveys at stated intervals will give precise information about natural succession under the prevailing climatic and edaphic conditions, and will furnish data on the means of dispersal of species in this region." [1]

A physical feature of little interest to the botanists but offering

[1] Lewis, I. F., "The Flora of Penikese, Fifty Years After," *Rhodora*, Vol. 26, No. 310, pp. 182–4, October, 1924.

a relatively important habitat for animals consists in the low stone fences that have been built in somewhat rambling fashion over the hills of the main island. Though now falling down in places, these walls harbor numbers of spiders, isopods and insects. The old houses and sheds are likewise inhabited by many insects and spiders.

Earlier Collecting on Penikese.

Although Penikese Island has been visited by naturalists from time to time during the past fifty years, only one paper has been encountered that deals with the fauna above the tide line. It may readily be understood why the pioneers of Agassiz's school had little time for study of the land fauna, engrossed, as they then were, with the revelations of marine life. Yet it seems worth while to quote in full the following communications from members of the original laboratory written fifty years after.

"I do not think that any one at Penikese listed the land snails, insects or other land animals except birds. We were busy with the novelties of the sea. C. O. Whitman gave most of his time to the birds, and I think had a list. F. H. Snow knew birds also. I made no formal list of the fishes, though I began my studies on them there. I could give an approximate record now. I listed also the Algæ, as well as the land plants. Walter Faxon recorded the marine Crustacea, and I made a list of the mollusks. Whether it is in existence now, I do not know. Time works havoc with manuscripts.

"Very sincerely yours,

"David Starr Jordan."

"I do indeed thank you for the pictures of Penikese and the laboratory at Woods Hole. I regret exceedingly that I cannot help you in the matter of Flora and Fauna of that Island. I was too busy with my lecture work to do more than that. The terns' nests with eggs were very abundant and a little work was done on its embryology.

"Faithfully yours,

"Edw. S. Morse."

Information of similar purport has been given me by Professor Cornelia M. Clapp, and Mr. Samuel E. Cassino, others who worked in the old laboratory on Penikese.

It is of particular interest that the only species described from Penikese (of which record has been found) is one of those relatively rare forms of crustacea, the branchiopods, that it should bear the name of the great teacher, and that the description should have been published in the second and final year of the original laboratory. The species is *Eulimnadia agassizii* Packard (1874);[1] it seems never to have been collected elsewhere.

In later years the island has been visited at various times by collectors and specimens have been deposited in several museums; but such collecting was done incidentally or in course of trips to various islands, and, almost invariably, without special publication. An exception is found in the paper by A. P. Morse entitled "Notes on the Orthoptera of Penikese and Cuttyhunk," appearing in *Psyche*, Vol. 7, p. 179, December, 1894.[2]

Among other naturalists who have submitted specimens from Penikese to various museums are J. P. Moore, Manton Copeland, Alexander Forbes, and J. T. Nichols. The following notes are quoted from a letter of Dr. Moore to the editor; "In 1892 and again in 1895, I collected in a small pond on Penikese two species of leeches, *Helobdella stagnalis*, and *Erpobdella punctata*. A few years later I again visited the island and think that I found leeches, but cannot find the record. Leeches are so easily carried by the aquatic birds that introduction is readily accomplished and one may expect to find other species. Even if all of the leeches should be destroyed by a prolonged period of drying of the small pools, the same or other species could be

[1] Packard, A. S. In Sixth Annual Report of the Peabody Academy of Science, Salem, Mass., 54, 1874.

In a later publication Packard says:

"About 100 females, mostly with eggs, occurred in a small pool of fresh water on Penikese Island, Buzzard's Bay, Aug. 27, 1873, collected by Mr. Walter Faxon. Upon examining the pool the following July or August (1874), the young, about a line in length, were found, but the pool subsequently dried up." (Quoted from "A Monograph of the Phyllopod Crustacea of North America, with remarks on the Order Phyllocarida," 12th Annual Report of the U. S. Geological and Geographical Survey of the Territories (for 1878), pages 295–590; Washington, 1883.

[2] I am endebted to Mr. C. W. Johnson for reference to the paper on Orthoptera. The species collected on Penikese are listed in another place. Editor.

quickly reëstablished. . . . There are probably other species of Oligochæta in addition to the three named in your list. I know that I found *Enchytræus albidus* (= *Halodrilus littoralis*) on the strand and one of the small Naiidæ in fresh water but these records also are not available just at present."

Discussion of Results.

As will later be shown in detail, animals of 216 species were taken and subsequently identified, and these, grouped by phyla and lesser subdivisions, are distributed as follows:

Number of Species of Animals Collected from Penikese Island, August, 1923.

Annelids	3	species
Mollusks	1	" [1]
Arthropods:		
Crustacea	2	"
Myriapods	1	"
Insects	162	"
Arachnids	15	"
Vertebrates:		
Amphibia	1	"
Reptiles	1	"
Birds (actually resting on the island)	29	"
Mammals	1	"
Total	216	"
Insects by Orders:		
Odonata	3	
Orthoptera	8	
Hemiptera (terrestrial 20, aquatic 6)	26	
Neuroptera	2	
Lepidoptera	13	
Diptera	47	
Coleoptera (terrestrial 30, aquatic 24)	54	
Hymenoptera	9	
Total number of species of insects	162	

It is worth while to allude to certain groups that are not represented in the list. Although a thorough search was made for fish in the brackish and freshwater ponds, none were found. Since isolation is not usually sufficient to account for the want of small fish in ponds, even such as may occasionally become

[1] An aquatic gastropod lost before identification.

nearly dry, the absence of such forms is doubtless to be attributed to the abundance of fish-eating birds on the island. It is notable in this connection that the amphibia were represented by a single species, the common toad, the least attractive member of the class to carnivorous animals. Among reptiles, too, turtles and water snakes must be extremely rare, if present at all, the only reptile taken being the common garter snake, of which several specimens were obtained both by the botanists on July 24, and by the zoölogists on August 7. Toads were also taken on both days. It appears that previous collectors interested in the herpetology of the island have found only the toad and the garter snake. It is surprising that aquatic crustacea were not found. Not even were copepods encountered in net collections from the small ponds. Perhaps in this respect, as in others, the results would have been different at another season.

Viewing the fauna of the island, as a whole, we observe that it is largely arthropodan—insect, arachnid and myriapod. Exclusive of arthropods and birds, we found but 4 species of invertebrates (3 earthworms and 1 gastropod) and 3 of vertebrates (an amphibian, a reptile, and a mammal). The invertebrate fauna would have been greatly extended by giving consideration to minute forms of animal life; but the vertebrate fauna was doubtless fully covered, except as to birds, which are migratory and seasonal. The apparent relative poverty of the island in nearly all forms of animal life (as regards number of species) may be attributed in great part to the very limited variety of environments, as afforded by plants and physical conditions.

An outstanding feature of the fauna of Penikese Island is its composition (almost entirely) of forms of very general distribution for the latitude. This, of course, is to be expected, since there are no known conditions upon the island to suggest the probability of the occurrence of unusual forms of life. In such a place we must seek not to find a fauna of special types, but rather to ascertain which of the common types of animal life encounter there the conditions favorable or requisite for their existence. Nevertheless, Penikese happens to be the place of collection of at least one specific type. (See p. 23 above.)

It is hoped that, quite apart from any interest which might

attach to this survey from its relation to the historical place and occasion, it will serve a useful scientific purpose as a basis of comparison with future conditions. It would be desirable for collections to be made at all seasons, under various weather conditions and in different years. Penikese Island has recently been set aside by the State of Massachusetts as a Bird Sanctuary, and placed under the Board of Conservation who are interested in fostering both the birds and the insect life of the island. The entire aspect of the insect fauna of the island may change materially at any time in consequence either of the invasion of new species adapted to present conditions but not yet arrived, or as a result of changing conditions of vegetation upon the island, such, for example, as would follow upon renewal of grazing or upon natural or artificial reforestation. In event of such change it will fortunately be possible, to some extent, to compare new and old conditions with respect to both plants and animals, and thus to study problems of coördination between the two kingdoms. We have, therefore, thought it a proper function of the introductory part of this report to direct attention to the forms that appear to be lacking, as well as to those that were found. Needless to say, future comparisons must not omit from consideration the season of the year and the precedent meteorological conditions such as are of record elsewhere than in this paper.

Animals of Land and Ponds, Penikese Island, August, 1923.

ANNELIDS.

(Identification by U. S. National Museum.)

Lumbricus terrestris Linnæus.

One adult and one apparently immature example taken near red house.

Helodrilus calignosus trapezoides (Savigny).

One example taken at same place.

Helodrilus tenuis (Eisen).

Two examples taken with preceding species and one in southern part of island.

MOLLUSCS.

(Gastropod from pond not identified.)

ARTHROPODS.

Crustacea.

(Identification by U. S. National Museum.)

Oniscus asellus Linnæus.

Porcellio rathkei Brandt. Abundant under boards and logs and in stone fences.

Myriapods.

(Identification by R. V. Chamberlain, Museum of Comparative Zoölogy, Cambridge, Mass., through U. S. National Museum.)

Lithobius forficatus Linnæus.

Insects.

Odonata.[1]

(Identification by C. B. and J. E. Wilson.)

Anax junius (Drury). Pond 1–*B*; three nymphs.
Pond 2–*B*; four nymphs.
Pond 1–*D*; seven nymphs.

Celithemis eponina (Drury); seen flying about in two places.

Ischnura verticalis (Say); Pond 2; two adults.
Pond 2; four adults.

Orthoptera.

(Identification by C. E. McClung.)

Gryllidæ.

Gryllus assimilis.

Nemobius vittatus Harr.

[1] In reports on aquatic insects (Odonata, Hemiptera, Coleoptera) the several small ponds are distinguished by letters and numbers, as follows: The two small water-holes near the northwest corner of the island are designated 1-*B* and 2-*B*, 1 being southwestward of 2; 1-*C* (Cattail Pond) is on the eastward side of the island near the keeper's house; near the southerly tip of the eastward extension of the island (Tub Point) are two small ponds, 1-*D* and 2-*D*, 1 being northeastward of 2. The largest pond, that near the southerly point of the main island, known to the collectors as South Pond (*A*) was decidedly brackish and yielded only one aquatic insect. The specific gravities (uncorrected for temperature) and the temperatures of the waters of these ponds were as follows: South Pond, 1.015 (20° C.); 1-*B* and 2-*B*, 0.99 (21° C.); 1-*C*, 0.99 (20° C.); 1-*D*, 1.0018 (23° C.); 2-*D*, 1.005 (22° C.).—EDITOR.

Locustidæ.

Conocephalus fasciatus De G.

Orchelimum vulgare Harr.

Acrididæ.

Melanoplus femur-rubrum De G.

Melanoplus atlanis Riley.

Chorthippus maculipennis Scudd.

Dissosteira carolina Linn.

Generally distributed, localized only by distribution of food plants.

The following list of Orthoptera appeared under the title "Notes on the Orthoptera of Penikese and Cuttyhunk," by A. P. Morse. (*Psyche*, Vol. 7, p. 179. Dec. 1894.) [1]

SPECIES COLLECTED ON PENIKESE.

Acrididæ.

Stenobothrus æqualis Scudd. Common.

Stenobothrus maculipennis Scudd. Common.

Chortophaga viridifasciata De G. Scarce.

Dissosteira carolina Linn. Common.

Psinidia fenestralis Serv. Scarce.

Melanoplus atlanis Riley. Scarce.

Locustidæ.

Xiphidium (*Conocephalus*) *fasciatum* De G. Common.

Gryllidæ.

Nemobius vittatus Harris. Common.

Hemiptera (Heteroptera).

(Identification by C. W. Johnson, Boston Society of Natural History.) (See also "Aquatic Hemiptera" below.)

Pentatomidæ.

Cænus delius Say.

Coreidæ.

Harmostes reflexulus Say.

Lygæidæ.

Cymus luridus Stål.

[1] Note by courtesy of C. W. Johnson.

Nabidæ.

Pagasa fusca Stein.
Nabis propinquus Reut.
Nabis ferus Linn.

Miridæ.

Teratocoris discolor Uhl.
Pœciloscytus basalis Reut.
Lygus pratensis Linn.
Onychumenus decolor Fall.

Saldidæ.

Saldula interstitialis Say.

Hemiptera (Homoptera).

(Identification by C. W. Johnson, Boston Society of Natural History.)

Cercopidæ.

Philænus leucophthalmus Linn. var. *ustulatus* Fall.
Philænus lineatus Linn.

Cicadellidæ.

Helochara communis Fitch.
Drœculacephala mollipes Say.
Drœculacephala minor Walk.
Acucephalus albifrons Linn.
Deltocephalus simplex Van D.

Fulgoridæ.

Liburnia pellucida Fabr.
Liburnia campestris Van D.

Aquatic Hemiptera.[1]

(Identification by C. B. and J. E. Wilson.)

Arctocorisa interrupta (Say); Ponds 1–*D* and 2–*B*; three specimens in all.

Belostoma flumineum Say; Pond 1–*B*; one nymph, identification doubtful.

Corixa verticalis Fieber: Pond 2–*D*; twenty-two specimens.
Pond 1–*D*; twenty specimens.
Pond 1–*B*; one specimen.
Pond 2–*B*; twenty specimens.
South pond; one specimen.

[1] See footnote to page 27.

Buenoa margaritacea Bueno; Pond 1–*D*; one doubtful nymph.
Gerris marginatus Say; Pond 1–*B*; ten specimens; Pond 1–*D*; one nymph.
Microvelia borealis Bueno; Pond 2–*B*; one specimen.

Neuroptera.

(Identification by C. W. Johnson, Boston Society of Natural History.)

Hemerobiidæ.
Hemerobius stigmaterus Fitch.
Chrysopidæ.
Chrysopa oculata Say.

Lepidoptera—Butterflies.[1]

(Identification by Manton Copeland, Preston S. Copeland and Frederick C. Copeland.)

Pieris rapae Linnæus (9).
Euvanessa antiopa Linnæus (1).
Vanessa atalanta Linnæus (1).
Vanessa huntera Fabricius (1).
Junonia cœnia Hübner (4).
Heodes hypophlæas Boisduval (5).

Lepidoptera—Moths.

(Identification by C. W. Johnson through Mr. Copeland.)

Hadena lignicolor Guenée (1).
Drasteria erechtea Cramer (3).
Drasteria crassiuscula Haworth (7).
Pyrausta fissalis Grote (1).
Crambus hortuellus Hübner (18).
Argyria nivalis Drury (1).
Ancylis Hübner sp? (1).

Diptera.

(Identification by A. H. Sturtevant, whose report is transmitted with the letter that is quoted immediately following.)

"I am enclosing the list of Penikese Diptera. There are 47 species on it—and these are all that I am able to identify with

[1] Figures in parentheses denote numbers of examples taken.

certainty. I should judge that there are about 30 additional species in the material, including the most conspicuous Dipteran encountered on the island, the mosquito, which apparently belongs in the genus Aedes, but which I have not included because the species remains very uncertain.

"There is one record that seems never to have been published as American, though the insect is so common that I suspect several people must have identified it correctly before—I have myself had the necessary information for two years now. One or two of the other records are of some interest from the point of view of geographical distribution, and that of *Muscina pascuorum* is apparently the only one for 1923 as early as August. Since the species appears to have been introduced from Europe only within the past few years any such data about it are of value, I think."

Asilidæ.

Asilus paropus Walker (Determined by C. W. Johnson).

Dolichopodidæ.

Dolichopus albicoxa Aldrich.
D. bifractus Loew.
D. pugil Loew.
D. splendidus Loew.

Lonchopteridæ.

Lonchoptera furcata Fallen.

Syrphidæ.

Allograpta obliqua Say.
Eristalis tenax Linne.[1]
Helophilus lætus Loew.
Platychirus quadratus Say.
Toxomerus marginatus Say.

Muscinæ.

Muscina pascuorum Meigen.

Stomoxyinæ.

Stomoxys calcitrans Linne.

Calliphorinæ.

Calliphora erythrocephala Meigen.

[1] Larvæ of this, or a very similar species were very common in samples of mud taken from the bottom of freshwater ponds.—R. E. C.

Lucilia cæsar Linne.
Lucilia sylvarum Meigen.
Pollenia rudis Fabricius.

Ortalinæ.

Chætopsis fulvifrons Macquart.
C. massyla Walker. These two species were taken in large numbers in the salt marshes, but no specimens were obtained of *C. ænea* Wiedemann and *C. apicalis* Johnson, which are the common salt marsh species of the mainland and of Naushon.

Trypaneinæ.

Euaresta bella Loew.
Trypanea abstersa Loew.
T. daphne Wiedemann.

Agromyzinæ.

Cerodonta dorsalis Loew.

Sepsinæ.

Sepsis violacea Meigen.
Themira minor Haliday.

Ochthiphilinæ.

Ochthiphila polystigma Meigen.

Chloropinæ.

Botanobia coxendix Fitch.
B. frit pusilla Meigen.
Chloropisca glabra Meigen.
Elachiptera costata Loew.
Melanochæta longula Loew.

Drosophilinæ.

Drosophila quinaria Loew.
Scaptomyza adusta Loew.

Ephydrinæ.

Dichaeta caudata Fallen.
Dimecænia spinosa Loew.
Ephydra subopaca Loew.
Notiphila scalaris Loew.
Ochthera mantis DeGeer.
Pelina truncatula Loew.

Psilopa atrimana Coquillett.
P. flavida Coquillett.
Scatella dichæta Loew.

Borborinæ.

Leptocera atra Adams. Common and widespread.
Leptocera brachystoma Stenhammar. This species does not seem to have been recorded as American. It is common under eel-grass at high tide level at least throughout the Woods Hole region. I have compared my specimens with British material determined by J. E. Collin, Esq.
Leptocera lutosa Stenhammar. Common and widespread.
Borborus geniculatus Macquart.

Phycodrominæ.

Cælopa parvula Haliday.

Coleoptera.

(Identification by C. W. Johnson, Boston Society of Natural History.)

Carabidæ.

Galerita janus Fabr.
Chlænius pennsylvanicus Say.
Harpalus caliginosus Fabr.
Harpalus compar Lec.
Harpalus pennsylvanicus DeGeer.
Harpalus herbivagus Say.

Staphylinidæ.

Ocypus ater Grav.

Silphidæ.

Necrophorus americanus Oliv.
Necrophorus marginatus Fabr.
Silpha lapponica Hbst.

Histeridæ.

Saprinus sp.

Cantharidæ.

Silis bidentatus Say.

Mordellidæ.

Mordellistena pustulata Melsh.

Elateridæ.

Melanotus fissilis Say.

Also a number of larvæ.

Melanotus communis Gyllenhall (Identified by U. S. National Museum).

Dermestidæ.

Dermestes vulpinus Fabr.

Nitidulidæ.

Conotelus obscurus Er.

Phalacridæ.

Stilbus apicalis Melsh.

Coccinellidæ.

Hippodamia parenthesis Say.

Coccinella novemnotata Hbst.

Tenebrionidæ.

Blapstinus mœstus Melsh.

Tenebrio obscurus Fabr.

Scarabæidæ.

Aphodius fimetarius Linn.

Xyloryctes satyrus Fabr.

Phyllophaga? sp.? (larvæ).

Chrysomellidæ.

Calligrapha elegans Oliv.

Galerucella notata Fabr.

Diabrotica duodecimpunctata Fabr.

Phyllotreta sinuata Steph.

Curculionidæ.

Brachyshynchus ovatus Linn.

Aquatic Coleoptera.[1]

(Identification by C. B. and J. E. Wilson.)

Acilius semisulcatus (Aubé); Pond 1–*D*; two specimens.

Agabus disintegratus Crotch; Pond 1–*B*; three specimens.

Berosus striatus (Say); Pond 1–*B*; two specimens.

Cœlambus impressopunctatus (Schall.); Pond 1–*D*; nine specimens.

[1] See footnote to p. 27.

Cælambus inæqualis Fabricius; Pond 1–*B*; six specimens.
Deronectes depressus Fabricius; Pond 1–*B*; ten specimens.
Dytiscus fasciventris Say; Pond 1–*C*; two specimens.
Hydrophilus obtusatus (Say); Pond 1–*B*; one specimen.
Haliplus ruficollis DeGeer; Pond 2–*B*; eighteen specimens.
Hydroporus americanus Aubé; Pond 1–*B*; three specimens.
Hydroporus modestus Aubé; Pond 2–*B*; twelve specimens.
Hydroporus niger Say; Pond 1–*B*; fourteen specimens.
Hydroporus proximus Aubé; Pond 1–*B*; two specimens.
Hydroporus stagnalis G. & H.; Pond 1–*B*; seventeen specimens.
Hydroporus tenebrosus Lecepède; Pond 1–*B*; one specimen.
Hydroporus undulatus Say; Pond 1–*B*; twenty specimens.
Octhebius foveicollis Lecepède; Pond 2–*D*; two specimens.
Philhydrus cinctus Say; Pond 2–*D*; fifty specimens.
Philhydrus nebulosus Say; Pond 1–*D*; one specimen.
Rhantus binotatus Harris; Pond 1–*D*; two specimens.
Rhantus bistriatus Bergst.; Pond 1–*B*; one specimen.
Thermonectes basilaris Harris; Pond 1–*D*; two specimens.
Tropisternus glaber Herbst; Pond 2–*B*; six specimens.
Tropisternus nimbatus Say; Pond 1–*C*; one specimen.

Hymenoptera.

(Identification by C. W. Johnson, Boston Society of Natural History.)

Ichneumonidæ.

Therion morio Fabr.

Formididæ.[1]

Myrmica scabrinodis Nyl. subsp. *schencki* Emery var. *emeryana* Forel.
Lasius niger Linn. var. *americanus* Emery.
Lasius (*Chthonolasius*) *brevicornis* Emery.

Halictidæ.

Halictus provancheri Cress?

Megachilidæ.

Osmia lignaria Say.

Apidæ.

Bombus fervidus Fabr.

[1] Identified for the Boston Society of Natural History by Dr. W. M. Wheeler.

Bombus americanorum Fabr.
Bombus bimaculatus Cress.

Arachnids.

(Identification by J. H. Emerton, through U. S. National Museum.)

Enoplognatha marmorata Hentz.
Epeira trivittata Keys. Many specimens.
Epeira pratensis Hentz. Immature.
Epeira stellata Hentz. Immature.
Argiope trifaciata Forskal. Young, several specimens.
Tetragnatha laboriosa Hentz.
Agalena naevia Walck. All young, half grown.
Dolomedes sexpunctatus Hentz. Young.
Lycosa helluo Walck. With egg cocoon.
Pardosa nigripalpis Em.
Pardosa glacialis Th.
Pardosa lapidicina Em.
Zelotes ater Hentz. Adult male and young.
Ariadne bicolor Hentz.
Phidippus multiformis Em.
Xysticus gulosus Keys.[1]

Birds.

Observed and identified by Ondess L. Inman and Everett C. Myers. (With confirmatory observations by others.)

Song Sparrow—*Melospiza melodia melodia.* Most common land bird.
Chipping Sparrow—*Spizella passerina passerina.* Few.
Seaside Sparrow—*Passerherbulus maritimus maritimus.* Common.
Sharp tailed Sparrow—*Passerherbulus caudacutus.*
English Sparrow—*Passer domesticus.* Fairly common about buildings.
Field Sparrow—*Spizella pusilla pusilla.* Few.
Vesper Sparrow—*Poocætes gramineus gramineus.*
Common Tern—*Sterna hirundo.* Most common.

[1] Specimens identified by J. H. Emerton through Boston Society of Natural History.

Roseate Tern—*Sterna dougallii.* Fairly common.
Least Tern—*Sterna antillarum.* Rare.
Cory Shearwater—*Puffinus borealis.* Rare.
Herring Gull—*Larus argentatus.* Rare.
Laughing Gull—*Larus atricilla.* Rare.
Meadow Lark—*Sturnella magna.* Few (6).
Yellow Warbler—*Dendroica æstiva æstiva.* One pair.
Upland Plover—*Bartramia longicanda.*
Semipalmate Sandpiper—*Ereunetes pusillus.* Very common.
Little Green Heron—*Butorides virescens.*
Ruddy Turnstone—*Arenaria interpres morinella.* Two.
Red-wing Blackbird—*Agelaius phœniceus.* Common.
Spotted Sandpiper—*Actitis macularia.*
Least Sandpiper—*Pisobia minutilla.*
Killdeer—*Oxyechus vociferus.*
Yellow legs—*Totanus* sp.? Rare.
Woodcock—*Philohela minor.*[1] One.
Virginia Rail—*Rallus virginianus.*
Curlew—*Numenius* sp.?
Eve Swallow?—
Ringneck Plover—*Aegialitis semipalmata* (Semipalmate Plover).

Other Vertebrates.

Bufo fowleri—Common toad. 4 examples.
(Identified by Miss Doris M. Cochran, U. S. National Museum.)
Thamnophis sirtalis sirtalis—Common garter snake. 6 examples.
(Identified by Miss Doris M. Cochran, U. S. National Museum.)
Peromyscus leucopus noveboracensis (Fischer). Field mouse. One example taken in one of the traps left on the island. Specimen preserved by the kindness of the State caretaker on Penikese; identified by F. E. Wood.

[1] Reported by D. B. Young.

THE CAUSE AND NATURE OF ENCYSTMENT IN *POLYTOMELLA CITRI.*

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Although encystment has long been known to play an important part in the life of most protozoa the cause and nature of the phenomenon has been the subject of relatively little careful experimental work. The evidence derived from the earlier work, slight as it was, together with the fact that cysts of protozoa are very resistant to drying, toxic substances, etc., has led the writers of text-books to speak of encystment as a passive response to adverse environmental conditions (Calkins 1909, Minchin 1912, Doflein 1916). In spite of the fact that such generalizations are quite common, there is little evidence that, on critical examination, cannot be interpreted otherwise than as mentioned above. The general confusion that has arisen from the conflicting data of almost every piece of work on this subject makes it desirable to give a brief historical review.

From the time that encysted protozoa were first observed in 1769 by Saussure until 1855 the few observations made on this subject did not deal at all with the cause of the process, but merely with establishing the relationship between cysts and the active forms to which they give rise. In 1855 Cienkowsky not only called attention to the fact that encystment is probably not universal among protozoa (as was supposed before that time) but also succeeded in producing encystment by a lengthy drying up of the medium.

After this date dessication was considered to be the principal, if not the sole cause of encystment, until the subject was attacked by Maupas and Fabre-Doumergue in 1888. The former produced encystment in predaceous ciliates by the deprivation of food, while the latter showed that drying up of the medium was not of paramount importance. By growing several ciliates in cultures placed in a moist chamber, he discovered that they

regularly encysted. This author attributed the causal factors to putrefaction of the medium, resulting in the release of toxic chemical substances.

The contention of Maupas has received support from other workers. Among them Root (1914) concludes that lack of food is the cause of encystment in the suctorian Podophrya collini. Mast (1917) obtained similar results with *Didinium nasutum*. He says: "Encystment in Didinia can usually be induced by cutting off the food supply. But it frequently occurs when there is an abundance of food present and sometimes it does not occur when there is none." However, Mast and Ibara (1923) after some carefully conducted experiments decide that "They encyst more freely in cultures supplied with food than in those without food, and this probably is due to greater increase in numbers, resulting in greater accumulation of waste material in the one than in the other." Hogue (1915) observed that the limax amœba upon which she was working encysted in the presence of abundant bacteria, upon which it feeds, and that the cell body of the encysting amœba always contained numerous bacteria. In both this paper and a later one (1917) she expresses the opinion that accumulation of byproducts of metabolism and oxygen deficiency are the principal causal factors. She thinks that they operate by causing the amœbæ to lose the power of assimilation. Carter (1919) concludes that abundant food is necessary for encystment of *Amœba proteus*.

Kofoid and Swezy (1921) consider that encystment in the marine dinoflagellates is due to one of two causes, the need for protection during the period of multiple and binary fission, and the need for a quiescent period for the assimilation of a large food body that has been ingested. Hall (1925) assigns *Oxyrrhis marina* to the second group given by Kofoid and Swezy. In addition, referring to drop cultures sealed with vaseline, he says: "After six weeks, exclusively non-motile forms were observed; in these, no flagella could be detected in observation under the oil immersion objective, and in some cases a 'cyst wall' seemed to surround the flagellates." Since no food vacuoles were present, he thinks that these non-motile forms may represent cysts formed in response to unfavorable conditions. From his

figures of cysts one would think that there should be no question of the presence of a cyst wall if one were there and it seems doubtful that they were really cysts.

Stolte (1922) reports that the ciliate *Blepharisma* encysts only in the presence of abundant food, and that large food vacuoles are present in those entering encystment. He also emphasizes the necessity for a relatively large number of *Blepharisma* in the culture before encystment is possible. This conclusion concerning a ciliate is quite in harmony with Kofoid and Swezy's for dinoflagellates.

Koffman (1924) suggests hydrogen-ion concentration as one of the essential factors in producing encystment in ciliates. He says: "The ciliates require ordinarily a definite P_h zone with a definite optimum, for their development. Below the minimum as well as above the maximum either initiates an encystment or the animals are destroyed."

In direct contradiction to the conclusions of the two preceding authors, we might mention the work of Brand (1923) who considers that, in the case of *Vorticella microstoma*, hunger, oxygen deficiency, and gradual drying up are the principal external factors for encystment. He relegates products of metabolism and chemical influence in general to a position of less importance.

Calkins (1915) found that *Didinium nasutum* regularly encysts entirely independent of adverse environmental conditions. However, encystment in this ciliate may not be entirely comparable to that of other forms, as a nuclear reorganization, similar to endomixis, occurs during the encysted phase. Fermor (1913) has described the same sort of reorganization in *Stylonychia pustulata*. With regard to the significance of encystment in *Didinium* Calkins says: "In addition to the casual encystment resulting from adverse environmental conditions, there is another form of encystment which involves more deep lying activities of the protoplasm," and again, "When the process is completed and the organisms emerge from their cysts they possess from five to seven times the vitality, measured by the division rate of the same race prior to encystment." In the face of this statement it is interesting to quote Mast (1917): "there was no evidence obtained indicating that conjugation or encystment has any

effect on death rate, fission rate, or variation in fission rate. This would indicate that neither of these processes is a rejuvenating process, at least not in the sense in which Calkins has used the term."

The foregoing review certainly indicates that more experimental work is needed on this subject, not only to obtain a better understanding of the protozoan life cycle, but also for the elucidation of the general biology of the so-called "resting forms of protoplasm." With this in view we have undertaken the work recorded in the following pages. The colorless phytomonad, *Polytomella citri* was selected as the organism to be experimented upon for two reasons: (*a*) it encysts very readily, and (*b*) most of the previous work has been confined to ciliates and amœbæ and we thought it desirable to extend the experimental field to flagellates.

We wish to express our gratitude to Professor E. G. Conklin, not only for his criticism, but also for his interest and encouragement. Our thanks are also due to Professor E. Newton Harvey for some very valuable suggestions.

Material and Methods.

The culture medium used in all of the experiments described in the following pages was made by boiling timothy hay in distilled water, about ten grams of hay to a liter of water. Three liters was the quantity usually made at one time, and all of the cultures of any one experiment contained medium of a single making, thus eliminating the possible error due to variation in the medium. Test tubes served the purpose of containers. Approximately 15 cc. was the quantity of medium in each culture.

Attempts were made to determine the effect of (1) various temperatures, (2) hydrogen-ion concentrations, (3) metabolic by-products, and (4) food deficiency on the tendency to encyst.

(1) The different temperatures were chosen because of their availability; and in spite of the fact that there was considerable variation in some cases the results were sufficiently striking for the present purpose. No attempt was made to arrive at an optimum temperature within a narrow range, as this was not thought to be necessary.

(2) For the work on hydrogen-ion concentration the ordinary hay infusion was used and the P_h was altered by either sodium hydroxide or acetic acid. The hydrogen-ion concentration was determined by the colorimetric method.

(3) The problem of metabolic by-products was approached in two different ways: first, by filtering the cultures through a porcelain filter and replacing the medium with fresh hay infusion, and, second, by removing the fluid from a thriving culture, in the same way, and replacing with medium taken from an old culture in which there were none active, all being encysted. In the one case the by-products were kept at a minimum, in the other they were increased beyond normal.

(4) To obtain a low food content in the cultures the hay infusion was replaced with distilled water and various percentages of distilled water and fresh hay infusion.

It has become rather customary, in recent years, to carry on work of this kind on organisms that have all been derived from a single individual. By this method one avoids comparing different strains, but, on the other hand, the results are applicable only to that particular strain, which probably can never be duplicated. The organisms used in the present work were all derived from a single culture, not from a single individual. It was thought, at the beginning, that by running a great number of cultures conclusions could be arrived at that would be applicable to the species, and that minor variations could be smoothed out by amount of data. The remarkable correspondence in the different cultures of any one series has entirely justified this procedure. This agreement has been so great that it has been possible to give only one result for all cultures of a series, with only two exceptions.

The condition of the cultures, in respect to active forms, is designated by the following terms: *very poor*, *poor*, *fair*, *good*, and *excellent;* and, with regard to cysts by: *very few*, *few*, *some*, *many*, and *very many*. These indefinite terms will doubtless arouse criticism. To this we can only reply that the differences in both active and encysted forms in the different series was so striking in all cases that they could easily be placed in their proper class, and, secondly, that it is the relationship which is of importance and not the actual numbers.

Polytomella was associated with bacteria and yeast in all of the cultures.

EXPERIMENTAL.

Temperature.—The procedure determined upon was to eliminate temperature first and then carry on the remainder of the experiments at the optimum temperature. Accordingly, thirty-six cultures were seeded and twelve of these were kept at each of the following temperatures: 10°–11° C. (series *A*1), 21°–22° C. (series *A*2), and 22°–27° C. (series *A*3). Series *A*3 was kept in the laboratory, which explains the wide variation in temperature. At the end of nine days all cultures of the three series were in excellent condition. Those of series *A*1 showed no cysts, *A*2 contained few, while some were found in *A*3. In fourteen days *A*1 was in good condition with no cysts; *A*2 was in fair condition, with some cysts; and *A*3 was poor, with many cysts. In twenty-one days *A*1 was only fair and no cysts had yet appeared; *A*2 had reached the same condition that *A*3 was in on the fourteenth day and *A*3 now contained no active forms and many cysts. The active forms had entirely disappeared from *A*2 by the thirty-fourth day. These two series continued in the same condition for the remainder of the experiment. On the thirty-fourth day *A*1 was in poor condition, without encystment. From the thirty-sixth to thirty-ninth day there was no ice in the refrigerator and the temperature rose from 10° to 21° C. At the end of those three days all twelve cultures were in fair condition and a few cysts had appeared in five of them. On the thirty-ninth day ice was again placed in the refrigerator and the experiment continued for twenty-one more days. At the end of that time there were practically no active forms in any of the cultures and the few cysts that were in five of the cultures on the thirty-ninth day were still present, but the remaining seven cultures had died out without any encystment whatever.

The first attempt showed that the cultures kept at 35° C. would not develop. Consequently, to attempt to determine the effect of so high a temperature, cultures were permitted to develop in the laboratory for a few days and were then placed in the incubator. It was found that at 35° thriving cultures would gradually die out until, at the end of two days, they would have com-

pletely disappeared without the formation of a single cyst. A little later twelve cultures were seeded and six of these were placed in the laboratory, the other six at a temperature of 30° C. In two days all were excellent cultures and many cysts had appeared in the ones kept in the laboratory, while none had encysted at 30°. On the third day all were alike, fair cultures with many cysts. The reason for these encysting more quickly than those of Series *A* will be explained later.

A second set of temperature experiments was started some time after series *A*. Series *B*1, *B*3 and *B*4 were kept at the same temperatures respectively as *A*1, *A*2 and *A*3. Series *B*2 was kept at 15°–22° C. With only slight variation the results were the same in series *B* as in *A*. Observations were made earlier than in *A* and it was found that the cultures kept at the lower temperatures, developed much more slowly than at the higher ones, such as laboratory temperature. Series *B*1 and *B*2 were still in rather poor condition on the seventh day while *B*4 was in good condition and a few cysts were present.

As noted above, a very few cysts appeared in five of the cultures of *A*1. This was not true for *B*1 as no encystment whatever occurred. The experiment was discontinued on the fifty-first day and seven cultures of series *B*1 had completely died out, the remaining five were in fair condition. These five were permitted to remain in the laboratory and in five days they had completely died out without any encystment.

We see that, as a whole, no encystment occurs at extremely low or high temperatures. Behavior at high temperatures differs from that at low in that, in going up the scale, encystment is not prevented until a point is reached which will kill the flagellates in a few days, while at low temperatures they develop more slowly and make very long lived and good cultures.

How are these results to be interpreted? At low temperatures one would expect the rate of metabolism to be lower and the time before encystment to be longer, but when the cultures died out without encystment a new light was thrown on the problem. If encystment were a response to adversely low temperatures we would expect it to occur there first. On the other hand, if encystment were a response to adversely high temperature we

would think that the two days which the cultures lasted at 35° C. would have been sufficient for the organisms to encyst rather than die. It is also noteworthy that the six cultures at 30° did not encyst until after their controls. It seems entirely justifiable to conclude that extremely low or high temperatures prevent encystment and that medium temperatures are not only conducive to rapid growth and division but also to encystment.

Hydrogen-ion Concentration.—It was noted in the above account that room temperature was the best one employed for the growth and division and encystment of *Polytomella citri*. Consequently, all of the remaining experiments were carried out at room temperature.

The procedure for the work on hydrogen-ion concentration has largely been given. Unaltered hay infusion (P_h 5.4) was used for the controls and, of course, these were not altered for the duration of the experiment. For the remaining cultures the P_h was restored to that at which the cultures were started on the third, ninth, and sixteenth days. Forty-eight cultures were seeded and the hydrogen-ion concentration was changed to the following P_h values (six cultures in each series): 3.5 (series *C*1), 4.5 (series *C*2), 5.4 (series *C*3), 6.2 (series *C*4), 7.5 (series *C*5), and 8.5 (series *C*6). Twelve cultures were kept as controls (series *C*7).

By the third day the cultures of series *C*1 were in fair condition and so they remained for the first week. After that the numbers decreased to such an extent that all could truly be classed as poor cultures. On the twenty-second day they were still poor and no cysts had been formed. Series *C*2, *C*3, *C*4, and *C*5, were practically identical throughout the first twenty-two days. This correspondence was so great that it is unnecessary to give separate results for each, which would be merely repetition. They were in good condition on the third day and excellent on the fifth. A very few cysts appeared on the fifth day. On the twenty-second day, the cultures were still in good condition and some cysts were present. Series *C*6 remained rather poor throughout the experiment and only a very few cysts were found on the twenty-second day.

The controls (*C*7) behaved quite differently from the other cultures. On the fifth day all twelve cultures of *C*7 were in

excellent condition and some cysts were present. By the eleventh day many cysts were found, while in the other series this condition was never reached. The cultures were only fair on the twenty-second day and very many cysts were present.

We now see that the cultures did excellently at any P_h from 4.5 to 7.5 and that encystment occurred with equal facility at any point within that range. However, in no experimental case was encystment as great as in the controls.

This experiment has been followed only to the twenty-second day, but observation was not discontinued at that time. They were examined on several occasions, but it will suffice to give only one of these, that made on the thirty-fourth day. All cultures were rather poor, so far as active forms are concerned and many cysts were present, though not as many as were found in the controls. This data, together with some which will follow, indicates that it was not the actual hydrogen-ion concentration which prevented the experimental cultures from equaling the controls in rapidity of encystment, but the sudden changing of the medium when the P_h was altered.

These results are quite contradictory to those of Koffman, and are more in harmony with Brand's conclusion that chemical influences are of little importance in producing encystment.

By-Products of Metabolism and Food Supply.

These two are taken up together partly because similar methods were used in dealing with them and partly because both factors entered into some of the experiments.

An attempt was first made to determine the effect of removing the by-products of metabolism by filtering the cultures through a porcelain filter and replacing the medium with fresh hay infusion. Twenty-four cultures were seeded and six of these had their culture medium replaced with fresh hay infusion every day (series $D1$) six others every second day (series $D2$), six others every third day (series $D3$) and the remaining six were kept as controls (series $D4$). The medium was not replaced in any of the cultures after the tenth day.

The results of this work are very startling. Whereas in all previous experiments the controls behaved beautifully, encysting

in about a week, the controls in this case did not encyst until the tenth day and then very few cysts appeared, most of the active forms dying rather than encysting. Although all the cultures of $D4$ (controls) were in excellent condition on the eighth day they had entirely died out by the fifteenth day and few cysts were present. The cultures of series $D2$ and $D3$ did not die out as soon as those of $D4$ nor did they encyst as quickly. On the twenty-second day of the experiment they had reached the same condition in which $D4$ was found on the fifteenth. By the thirtieth day all active forms had disappeared from the cultures of $D1$ and slight encystment had occurred.

Not knowing what to make of this behavior, twenty-four more cultures were seeded and the experiment repeated. These cultures were divided into four series $E1$, $E2$, $E3$, and $E4$, each series corresponding to the similarly numbered series of D. This yielded identically the same results as D with the exception that $E1$ and $E2$ died out without any encystment whatever.

It is well to state here that D was seeded from a culture of $C1$ shortly before it completely died out and E was seeded from a culture of $C2$ after it had reached a very poor condition. No cysts were transferred, only active individuals. Series C had likewise been seeded from $A3$. In an attempt to find the explanation for this loss of ability to encyst on the part of series D and E, some cysts were taken from $C6$, placed in fresh hay infusion and permitted to excyst. On the next day eighteen cultures were seeded from these newly excysted ones and two days later it was found that every one of the eighteen contained many cysts. As a check on this, cysts were taken from $C7$, washed in seventy per cent. alcohol, and permitted to excyst in fresh hay infusion. From these, thirty-six cultures were seeded and on the second day they were found to have repeated the activity of the eighteen, being largely encysted. In addition, cysts were taken from $E3$, washed in alcohol, excysted, and twelve cultures seeded from them. At the end of two days many cysts were found in all of the cultures.

By putting the last two paragraphs together, one gets a strong indication that the longer *Polytomella citri* is removed from encystment, the less is their tendency to encyst.

To carry this further, series *F* was started. *F*1 (six cultures) was seeded from *A*1 on the sixtieth day after that series was seeded; *F*2 (six cultures) was seeded from *E*2 on the eighteenth day; *F*3 (six cultures) was seeded from active ones that were ten days removed from newly encysted individuals; and *F*4 (eighteen cultures) was seeded from active ones excysted only five days previously.

On the third day all cultures were excellent, with the exception of *F*1, which were in fair condition. No cysts had appeared in the first three series, but some were present in the cultures of *F*4. By the seventh day *F*1 and *F*2 were in excellent condition, with no cysts while the cultures of *F*3 were in good condition with some cysts and *F*4 was only fair with many cysts. Examination on the nineteenth day revealed the fact that the cultures of all four series were very poor. A very few cysts had appeared in several of the cultures of *F*1; there were none at all in *F*2 while *F*3 and *F*4 contained many cysts.

It is now clearly seen that the longer encystment is prevented, whether by low temperature (*F*1) or by long continued transfer (*F*2), the less is the tendency to encyst, and if this procedure be sufficiently extended the ability to encyst is entirely lost. The morphological basis for this is given in the next section.

It was determined to repeat the work of *D* and *E*, using newly excysted forms. Accordingly twelve cultures were seeded and six of these had their medium replaced every day, the others were kept as controls. In two days the controls were quite generally encysted while those filtered every day showed no cysts until the third day.

Some cultures of the types found in *F*2 and *F*4 were filtered and the medium replaced with fluid taken from cultures that had entirely encysted. In the former no encystment whatever occurred, while in the latter it was slowed up, usually for about one day, as compared to the controls. When first placed in such a medium, we were surprised to find that division was apparently stimulated, as examination under the microscope would reveal a great many late division stages, which are the only ones that can be detected in living material. Although encystment was slightly deferred in the case of those of type *F*4, the cultures

themselves in all instances ceased to show any active forms before they disappeared in the controls. This was probably due to the lack of food.

Now let us inquire why *E*1 and *E*2 did not encyst at all. Was it because of the removal of the by-products of metabolism? The evidence is very much against such a conclusion. If that were true, we should have expected the cultures of the last mentioned experiment to have encysted before the controls, since in that case metabolic by-products were probably increased. Also, in the case of the cultures seeded with newly excysted individuals in which the medium was replaced with fresh hay infusion every day, we should have expected encystment to have been postponed more than it was, if metabolic by-products were an important factor for encystment. Again in series *E*1 and *E*2 active forms remained in the cultures for ten days after filtering was stopped. This, we think, would have been sufficient time for the necessary by-products to accumulate, yet no encystment occurred. The arguments just recounted seem to entirely justify the conclusion that the filtering of a culture and replacing its medium with fresh hay infusion defers encystment, but this effect is produced by the stimulus to growth and division given the organisms by changing the medium suddenly, and is not due to the removal of an adverse environmental factor created by the presence of metabolic by-products. This is quite comparable to seeding a new culture.

The reader will doubtless have thought of the fact that an ever plentiful supply of food was present during the early course of *D*1, *D*2, *D*3, *E*1, *E*2, and *E*3. However, the same arguments that applied to metabolic by-products will apply here and we can likewise conclude that food deficiency is not the cause of encystment. This was carried further by filtering thriving cultures and replacing their medium with distilled water, 25 per cent. hay infusion and 75 per cent. distilled water, etc. to pure hay infusion, a change of 25 per cent. in each step. In the cultures that received pure distilled water no encystment took place and the cultures died out in two days. In the others, however, neither fission nor encystment seemed to be materially affected.

A few experiments were carried out which may have involved

oxygen deficiency. Thriving cultures of Polytomella were sealed in glass tubes, very little air space being left between the top of the medium and the sealed end. Since no photosynthetic organisms were present, it would seem as though the dissolved oxygen of the medium should have decreased. At the end of one week the cultures had died out and very few cysts had been formed.

MORPHOLOGICAL CHANGES ACCOMPANYING THE EXPERIMENTS.

When a hay infusion culture is seeded with *Polytomella citri* taken from a stock culture (one containing uncooked hay in distilled water) the organisms will multiply rapidly for a few days, during which time staining with iodine will indicate the presence

FIG. 1. Diagrammatic figures traced from photomicrographs of Polytomellas stained with iodine. Solid black indicates starch. Equal magnification. (See text for further explanation.)

of relatively little starch (Fig. 1*a*). At the end of from two to five days it will be noticed that the starch increases to such an extent that the entire cell seems filled with it (Fig. 1*b*). It is at this time that encystment occurs. Polytomella never encysts except when the cell-body is filled with starch, and, on the other hand, such a culture has never been found to be unaccompanied by cysts.

As stated above, all cultures used in this work were derived from a single stock, and later cultures were seeded with active ones taken from previous experiments.

It was not only found that when *Polytomella* is so transferred from culture to culture for some time their tendency to encyst becomes practically negligible, but also that marked morphological changes occur. When encystment is thus prevented for some time the organism will frequently show considerable decrease in size, almost invariably the cytoplasm will become coarsely vacuolated, and the starch entirely disappear. Fig. 1*c* was taken from series *E*4 on the fourteenth day after seeding; Fig. 1*d* from series *A*1 on the sixtieth day. In series *D*1, *D*2, *D*3, *E*1, *E*2, and *E*3, it was noticed that all contained little starch. In living mounts the cell-body was quite clear and the size above normal. Fig. 1*e* was taken from *E*2 on the eighteenth day. The abnormally large size was lost as soon as the numbers began to decline. Eventually all of these cultures showed only a few degenerate, vacuolated individuals.

It was thought desirable to determine whether or not these degenerate forms could give rise to healthy cultures. Accordingly, series *F* was started. The history of these cultures has already been given, with the exception of the data found in Fig. 2. Fig. 2*a* was made from a typical individual taken from

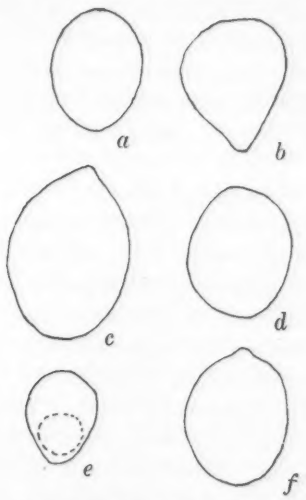

FIG. 2. Camera-lucida outlines showing variations in size. Figure e vacuolated. (See text for explanation.) Magnification 1825 ×.

the culture which furnished the starting point for F_4; Fig. 2*b* was taken from F_4 two days later. Fig. 2*c* was taken from E_2 on the day that F_2 was seeded; 2*d* was taken from F_2 two days later. Fig. 2*e* was taken from A_1 on the day that F_1 was seeded; Fig. 2*f* from F_1 on the second day. A glance at these figures will show that transference of these degenerate forms to a new and favorable medium results in a return towards normality, with the exception of the formation of starch, as F_1 and F_2 formed little of that material.

Discussion.

The Causes of Encystment.—In the preceding section attention was called to the fact that *Polytomella citri never encysts except when the cell body contains abundant starch.* Since this is so it seems as though the search for the external factors for encystment becomes resolved into a search for the factors which encourage the formation and storage of starch. Adverse conditions of the environing medium certainly would not be conducive to the formation of this material, at least the above experiments so indicate, and, consequently, we need not be surprised at the conclusion drawn from the present work, namely, *Polytomella citri* encysts only under such conditions as are favorable for growth and reproduction, and adverse conditions lead to death of the culture rather than encystment. This very thing, however, may prolong the life of a culture, so far as active forms are concerned.

The presence of abundant starch in *Polytomella* as a prerequisite for encystment is quite in harmony with the observations of Hogue on *Amœba limax*, Carter on *Amœba proteus*, Kofoid and Swezy on marine dinoflagellates, Stolte on *Blepharisma*, Mast and Ibara on *Didinium*, and Hall on *Oxyrrhis marina*. In fact, most of the recent work indicates that abundant food is much more conducive to encystment than is starvation, which was formerly supposed to be one of the principal causes of encystment, and is still so given in text-books.

In the introduction a quotation was given from Koffman (1924). From this it is seen that altering the P_h to a lethal point either resulted in death or encystment, generally death.

From this he concludes that hydrogen-ion concentration is probably one of the most important factors in producing encystment. It seems to us that he would have been justified in concluding that adverse hydrogen-ion concentration might slightly speed up the process, if the internal condition of the protozoan disposed it towards encystment. It is just such conclusions as Koffman's that have led to an over-emphasis of the importance of adverse environmental factors in producing encystment and have led to some authors speaking of the phenomenon as a "passive adaptation to adverse environment." Although that may be true in a few instances, a critical review of the literature certainly does not indicate that it is the general rule.

The Nature of Encystment.—In a former paper Kater has shown that extensive changes occur during the encysted stage of *Polytomella citri*. This does not particularly involve the nucleus, the budding off of the new centriole and basal granule from the karyosome being the only possible regenerative change that was found. The most striking changes take place in the cytoplasm. The starch rapidly disappears when this resting phase is entered, and, coincident with its disappearance, the metachromatic granules are formed in great abundance. These are absorbed before excystment occurs.

Before attempting a discussion of the meaning of encystment in this flagellate, we must decide why prevention of encystment for several months results in loss of the tendency to store starch, and, consequently, to encyst. On a priori grounds we can say that it is either due to selection, direct effect of the environment, or a combination of the two. Although we may not be able to answer this conclusively, the present evidence indicates that selection does not enter into the matter. If it were due to selection of an encysting strain we should not expect cysts taken from $E3$ and $C7$ to give rise to cultures that would encyst in exactly the same time, two days, yet such is the case. $C7$ encysted much more quickly than did $E3$ and, if there were anything in the strain, the offspring should behave likewise. In the second place, $F1$ showed only a very slight tendency to encyst, mostly dying. Since these cultures were seeded from

*A*1 on the sixtieth day we must suppose, if it is a matter of selection, that the non-encysting variations are more viable than the encysting. This does not seem reasonable, because if it were so we would hardly expect any existing *Polytomella* to exhibit the phenomenon.

No attempt has been made to accurately measure the division rate in a non-encysting strain, such as *F*2, as compared to an encysting, *F*4. Mass observations lead us to believe that even when *Polytomella* has reached a condition of morphological degeneracy and may have entirely lost the power to encyst when they are transferred to a fresh and suitable medium, they are stimulated to a perfectly normal period of growth and division, with the exception that it does not end in encystment.

The morphological degeneration observed in the cultures *A*1, *B*1, *D*, *E*, etc., is very similar to that noted by Maupas in *Stylonychia* when conjugation was prevented and by Calkins in *Uroleptus*. We will not enter into the propriety of terming this senescence, and encystment as a rejuvenating process.

SUMMARY.

1. *Polytomella citri* encysts only when the cell-body contains considerable starch.

2. Encystment is not due to any perceptibly adverse environmental factors.

3. Optimum conditions for growth and reproduction are concomitant with maximum encystment.

4. Prevention of encystment, either by continuous transfer or by low temperature, if carried to sufficient extent, will result in morphological degeneracy and loss of the tendency to store starch and to encyst.

LITERATURE.

Allman.

'72 On Some Points in the Development of Vorticellidæ. Quart. Jour. Micr. Science, 2d series, 12.

Auerbach, L.

'54 Ueber Encystierung von Oxytricha pellionella. Zeitsch. f. wiss. Zool., 5.

Brand, Th.

'23 Die Encystierung bei *Vorticella microstoma* und hypotrichen Infusorien. Arch. f. Protist., 47.

Calkins, G. N.

'09 Protozoölogy.

'15 *Didinium nasutum*. 1. The Life History. Jour. Exp. Zoöl., 19.

'16 General Biology of the Protozoan Life Cycle. American Naturalist, 50.

Carter, L. A.
'19 The Cysts of *Amœba proteus*. Proc. Roy. Phys. Soc. Edinburg, 19.
Cienkowsky.
'55 Ueber Cystenbildung bei Infusorien. Zeitsch. wiss. Zool., 6.
Doflein, F.
'16 Lehrbuch der Protozoenkunde. Jena.
Fabre-Domergue.
'88 Recherches sur les Infusoires Ciliés. Annales des Sciences Naturelles, 7th srs., 5.
Fermor, X.
'13 Die Bedeutung der Encystierung bei *Stylonychia pustulata*. Zool. Anz., 42.
Goodey, T.
'13 The Excystation of *Colpoda cucullus* from its Resting Cysts and the Nature and Properties of the Cyst Membranes. Proc. Royal Soc., London, 86*b*.
Green Wood, M.
'96 On the Structural Changes in the Resting Nuclei of Protozoa. Part 1. The Macronucleus of *Carchesium polypinum*. Jour. of Physiology, 20.
Hall, R. P.
'25 Binary fission in *Oxyrrhis marina*. Univ. Cal. Pub. Zoöl., 26.
Hogue, Mary J.
'15 Studies in the Life History of an Amœba of the *Limax* Group. Arch. f. Protist., 35.
'17 Effect of Media on the Shape of *Amœbæ*. Jour. Exp. Zoöl., 22.
Kater, J. McA.
'25 Morphology and Life History of *Polytomella citri* sp. nov. BIOL. BULL., 49.
Koffman, M.
'24 Über die Bedeutung der Wasserstoffionenkonzentration fur die Encystierung bei einigen Ciliatenarten. Arch. Mikr. Anat., 103.
Kofoid and Swezy.
'21 The Free-Living Unarmored Dinoflagellates. Mem. Univ. Calif., 5.
Minchin, E. A.
'12 Introduction to the Study of the Protozoa.
Maupas, E.
'88 Recherches Expérimentales sur la multiplication des Infusoires ciliés. Arch. de Zool. exper., 2d series, 6.
Mast, S. O.
'17 Conjugation and Encystment in *Didinium nasutum*. Journ. Exp. Zoöl., 23.
Mast and Ibara.
'23 Encystment in *Didinium nasutum*, BIOL. BULL., 45.
Rhumbler, L.
'88 Die verschiedenen Cystenbildungen und die Entwicklungsgeschichte der holotrichen Infusoriengattung Colpoda. Zeitschr. f. wiss. Zool., 46.
Root, F. M.
'14 Reproduction and Reactions to Food in the Suctorian, *Podophrya collini* (n. sp.). Arch. f. Protist., 35.
Stein, F.
'49 Untersuchungen Uber die Entwicklung der Infusorien. Arch. f. Naturgesch. 15 Jahrb., 17.
Stolte, H. A.
'22 Verlauf, Ursachen und Bedeutung der Enzystierung bei Blepharism. Verhandl. d. dtsch. Zool. Ges., 1922.

SEQUENCE OF CORRESPONDING THIRD-CHROMOSOME GENES IN *DROSOPHILA MELANOGASTER* AND *D. SIMULANS*.

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It has been shown (Sturtevant, 1921*a*, *b*) that *Drosophila melanogaster* and *D. simulans* may be crossed, and accordingly it is possible to test suspected corresponding genes in the two species. There is, for example, a recessive vermilion-like eye-color (known as scarlet) in each species. If scarlet of either species is crossed to the wild type (or to any other eye-color) of the other species, the resulting hybrids have the wild-type or "red" eye-color. Therefore each species carries the dominant wild-type allelomorph of the scarlet mutant of the other species. But if scarlet *melanogaster* is crossed to scarlet *simulans*, the resulting hybrids have scarlet eyes. Therefore the two scarlets must represent modifications of the same wild-type gene, or at least of wild-type genes that have like effects on development. Such genes as the two scarlets are described in this laboratory by the term "corresponding," rather than (as formerly) "allelomorphic"—since it seems desirable to reserve the latter term for genes that regularly separate at maturation. In the present case the fact that all the hybrids are sterile prevents any test of the segregation.

A study of thirteen corresponding genes in the X-chromosomes of *melanogaster* and *simulans* (Sturtevant 1921*a*, and unpublished data) shows that the sequence of the loci concerned is the same in the two species. But it was shown by Sturtevant (1921*c*) that the sequence is not identical in the third chromosomes. If the three corresponding genes scarlet (st), peach (p), delta (Δ, formerly called "deltoid" in *simulans*) are mapped, the sequence is found to be stpΔ in *melanogaster*, stΔp in *simulans*.

The present paper is a report on three additional pairs of corresponding genes in this chromosome, which make it possible

to compare the maps in more detail. The three loci concerned, already well known in *melanogaster*, are sepia, hairless, and claret. In the cases of sepia and claret the mutant types of *simulans* are closely similar in appearance to the types of the same names in *melanogaster*, and it was a simple matter to obtain sepia hybrids and claret hybrids by crossing the types concerned. The evidence that these are corresponding genes is thus of the same nature as in the cases of scarlet, peach, and the sex-linked genes previously reported (Sturtevant, 1921*a*, *b*).

Hairless is, however, dominant in both species, so that the same difficulty was encountered here as in the case of delta—the hybrids show the mutant character even when it is introduced from only one parent. But in each species hairless, like delta, is lethal when homozygous; accordingly this lethal effect was used to test the correspondence of the hairless genes. Since the earlier tests of delta were not as clear-cut as might be desired, the new tests were carried out with flies containing both genes, and served to show that both loci correspond.

D. melanogaster females that were Δ/H—i.e., carried delta in one third chromosome and hairless in the other one—were crossed to *simulans* males that were also Δ/H. There resulted 169 hybrid offspring: 164 delta hairless, 4 delta, 1 hairless, 0 wild-type. If the delta genes do not correspond, so that Δ/Δ is viable, the cross should give half as many delta flies as delta hairless; if the hairless genes do not correspond, the hairless class should also be half as large as the delta hairless class. The few delta and hairless offspring produced are evidently due to crossing over in the mothers, 1.5 per cent. of whose eggs are expected to carry neither Δ nor H. The result was checked by crossing *melanogaster* females that were ΔH/+ by *simulans* males that were Δ/H. From this cross, all the ΔH eggs should give inviable zygotes, and equal numbers of delta and hairless offspring should result. There were obtained the following hybrids: 1 delta hairless, 225 delta, 226 hairless, 0 wild-type. The one delta hairless remains unexplained, for in this case the mothers carried C_{III}, which should have prevented all crossing over between Δ and H. This individual probably represents a new mutation, though it is possible that it was really homozygous

for Δ or for H, since the somatic effects produced by these genes are in many respects on the same characters but in opposite directions, so that heterozygosis for one of them might conceivably tend to counteract the lethal effect of the other one. Since all the *melanogaster-simulans* hybrids are sterile, it was not possible to test this exceptional individual. In any case, this one specimen cannot vitiate the conclusion that both delta and hairless are corresponding genes in the two species.

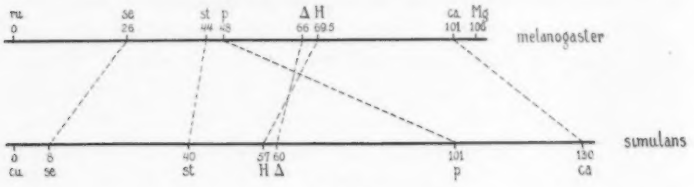

FIG. 1.

Fig. 1 shows the linkage relations of the six corresponding genes in the two species, together with the left-most and right-most known loci of each species.

The *melanogaster* map is based on the values given by Bridges and Morgan (1923). The simulans map is based on unpublished data, including counts from crosses involving several genes not here recorded. The intervals in this map are not yet accurately determined, because of a lack of favorable mutations; but they are more likely to be lengthened than to be shortened by further data. The sequence of the corresponding genes in simulans is clearly established, as the following data will show. Claret cannot be distinguished somatically from peach, so its position

TABLE I.

$\frac{+\,st\,+}{se\,+\,\Delta}$ ♀ × se st + ♂.

Non-Crossovers.	Single Crossovers.		Double Crossovers.	Total.
0.	1.	2.	1, 2.	
st se Δ	Δ sest	st Δ se	+ sest Δ	
121 142	70 44	43 79	21 8	528

has been determined with respect to minute, which is very close to it.

TABLE II.

$\frac{+\ H\Delta\ +}{st\ +\ +\ p}$ ♀ × st + + p♂.

0.		1.		2.		3.		1, 2.		1, 3.		2, 3.		1, 2, 3.		T.
HΔ	stp	p	stHΔ	Hp	stΔ	HΔp	st	Δ	stHp	+	stHΔp	H	stΔp	Δp	stH	
320	330	69	75	7	11	181	181	3	0	41	29	3	1	0	1	1,252

Minute (which is dominant) sometimes overlaps wild-type in appearance; accordingly only the minute offspring have been recorded in Table III. In the experiment reported in Table IV.

TABLE III.

$\frac{+\ +\ +\ +}{st\Delta pM}$ ♀ × st + p + ♂.

0.	1.	2.	3.	1, 2.	1, 3.	2, 3.	1, 2, 3.	T.
stΔpM	ΔpM	pM	M	stpM	stM	stΔM	ΔM	
126	48	77	51	22	25	28	4	381

TABLE IV.

$\frac{+\ \Delta\ +\ M}{st\ +\ ca\ +}$ ♀ × st + ca + ♂.

0.		1.		2.		3.		1, 2.		T.
ΔM	stca	stΔM	ca	stM	Δca	stcaM	Δ	M	stΔca	
39	19	4	2	24	18	0	2	8	3	119

this overlapping seemed to be slight, so all the offspring are recorded. It is possible, however, that the two M-ca crossovers may be due to such overlapping. More extensive counts from M/ca females, in experiments in which no other loci were studied, have not given any crossing over. There is an unusually low scarlet delta crossover value shown in Table IV., but the count is so small that no special significance is to be attached to this fact.

There can now be no doubt that one half or more of the right

limb of the third chromosome (in terms of map distance) has been inverted in one or the other species—since there is no clue as to which is the original sequence. The left-hand limit of the inverted section lies between scarlet and peach of *melanogaster*, which is presumably very close to the spindle-fiber attachment, and is certainly in a region where the mutant genes are mapped very close together and where coincidence of crossing over is very high (Bridges and Morgan, 1923). It will be interesting, as more mutant genes are studied in *simulans*, to see how this same material behaves when it lies further from the middle of the chromosome.

In the earlier account (Sturtevant, 1921*c*) it was suggested that the rearrangement of parts demonstrated for the *melanogaster* and *simulans* third chromosomes might be explained as resulting either from a duplication or from an inversion of a section. The new data here reported speak strongly for the inversion interpretation.

One might surmise that this inversion of a piece of a chromosome is the fundamental factor in the specific difference between *melanogaster* and *simulans* and the sterility of their hybrids. But it has been shown (Sturtevant, 1920) that at least some of the factors concerned in the inviability of certain classes of the hybrids are in the X chromosomes, where there has certainly been no such inversion.

This case shows that identity of sequence is not a necessary condition for correspondence of genes in different species. It also suggests that we need not be surprised should such an inversion be found within a species.

BIBLIOGRAPHY.

Bridges, C. B., and T. H. Morgan.

'23 The Third-chromosome Group of Mutant Characters of *Drosophila melanogaster*. Carnegie Inst. Wash., publ. 327, 251 + 10 pp., 3 plates.

Sturtevant, A. H.

'20 Genetic Studies on *Drosophila simulans*. I. Introduction. Hybrids with *D. melanogaster*. Genetics, *5*: 488–500.

'21*a* Genetic Studies on *Drosophila simulans*. II. Sex-linked Group of Genes. Genetics, *6*: 43–64.

'21*b* Genetic Studies on *Drosophila simulans*. III. Autosomal Genes. General discussion. Genetics, *6*: 179–207.

'21*c* A Case of Rearrangement of Genes in *Drosophila*. Proc. Nat. Acad. Sci., *7*: 235–237.

SYSTEMIC AND SEX DETERMINANTS OF BONE GROWTH (*MUS NORVEGICUS ALBINUS*).

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Studies of the chemical differentiation of bone during growth ('25*a*, *b*, *c*) and a comparison of bone growth in length with that in weight ('25*d*) indicated, as might be expected, that systemic and sex factors are participants in the determination of the mode and rate of development of this type of structure. It is therefore pertinent to attempt an evaluation of the influence of these factors in order that knowledge in this direction be extended and rendered more precise. Such has been done for the chemical differentiation and has been reported in the papers cited. This paper deals with their evaluation in the growth of the bones in weight and length.

The raw data were obtained from the same source as that which served for the previous studies, and hence are directly related thereto. The material analyzed consisted of the lengths and weights of the humerus and femur of male and female albino rats 23, 30, 50, 65, 75, 100 and 150 days of age, together with the respective body lengths and weights. The mean values and their probable errors are given in Table I.

The distribution of the material has been given ('25*a*) and need not be repeated. The animals were healthy. They came from the Experimental Colony stock of the Wistar Institute, had a common inheritance, and were raised under like conditions of diet and environment as described by Greenman and Duhring ('23).

Data on the osseous system of the albino rat on age have been reported by Jackson and Lowery ('12) and Donaldson and Conrow ('19). Their records do not lend themselves to an analysis, such as the present, because of the lack of systemic or sex separation.

TABLE I.

THE MEAN BONE AND BODY LENGTH AND WEIGHT AT THE STATED AGES.

Male.

	Length.			Weight.		
	Humerus.	Femur.	Body.	Humerus.	Femur.	Body.
Day.	Mm.	Mm.	Mm.	G.	G.	G.
23	13.4 ± 0.06	14.8 ± 0.09	95.3 ± 0.74	0.0557 ± 0.0020	0.0841 ± 0.0035	27.3 ± 0.62
30	15.7 ± 0.08	18.4 ± 0.11	116.1 ± 0.83	0.0786 ± 0.0021	0.1373 ± 0.0041	41.4 ± 0.81
50	18.3 ± 0.08	22.7 ± 0.13	143.2 ± 0.59	0.1018 ± 0.0016	0.2069 ± 0.0027	74.5 ± 1.80
65	21.2 ± 0.15	27.0 ± 0.24	169.3 ± 1.15	0.1488 ± 0.0035	0.3255 ± 0.0085	120.8 ± 2.05
75	21.9 ± 0.10	27.9 ± 0.22	174.8 ± 1.17	0.1591 ± 0.0028	0.3407 ± 0.0060	133.2 ± 3.24
100	22.9 ± 0.27	29.7 ± 0.33	182.5 ± 1.60	0.1847 ± 0.0055	0.4050 ± 0.0131	162.3 ± 4.60
150	25.9 ± 0.27	33.9 ± 0.33	205.8 ± 1.60	0.2556 ± 0.0041	0.5811 ± 0.0198	244.3 ± 6.90
				Female.		
23	14.0 ± 0.07	15.5 ± 0.10	98.9 ± 0.84	0.0573 ± 0.0017	0.0885 ± 0.0034	28.5 ± 0.70
30	15.3 ± 0.06	17.9 ± 0.09	110.8 ± 0.63	0.0725 ± 0.0015	0.1258 ± 0.0031	38.7 ± 0.66
50	18.2 ± 0.10	22.6 ± 0.16	141.8 ± 1.04	0.1008 ± 0.0019	0.2011 ± 0.0042	74.2 ± 0.79
65	20.5 ± 0.13	26.1 ± 0.17	161.8 ± 0.90	0.1333 ± 0.0023	0.2866 ± 0.0042	105.0 ± 1.55
75	21.2 ± 0.15	27.0 ± 0.21	166.8 ± 1.34	0.1417 ± 0.0037	0.3080 ± 0.0077	115.8 ± 2.56
100	22.5 ± 0.15	29.0 ± 0.23	177.1 ± 0.90	0.1732 ± 0.0031	0.3818 ± 0.0090	137.6 ± 2.20
150	24.0 ± 0.13	31.0 ± 0.30	188.4 ± 1.30	0.2033 ± 0.0050	0.4502 ± 0.0119	174.7 ± 4.40

Since growth is a process it should be studied as such and expressed in terms of rate. This point of view governs the present inquiry.

From Table I. it is seen that the femur is heavier and longer than the humerus at all ages in both sexes. This is a consequence of the fact that the rate of growth in weight and length (grams

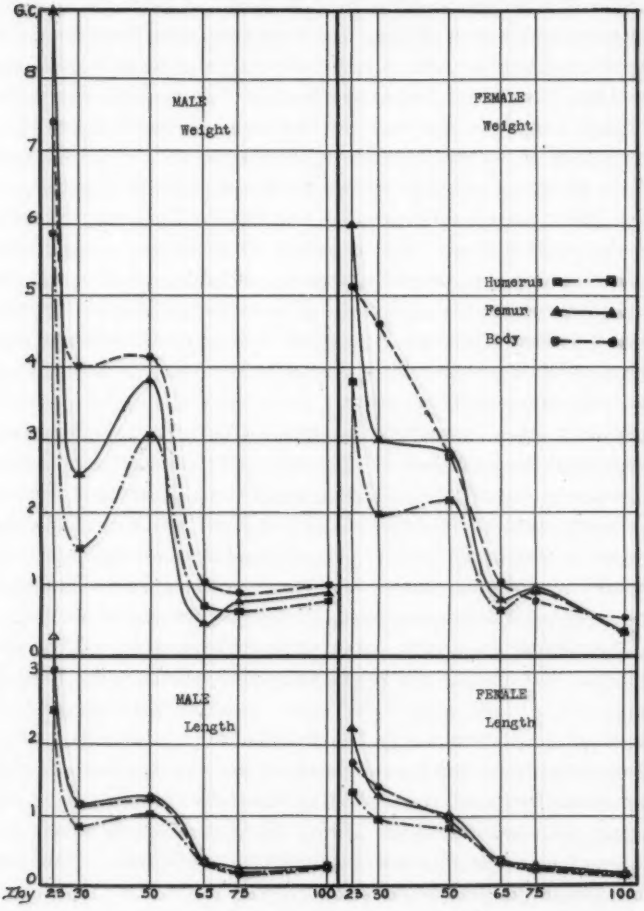

CHART I. The growth capacity in weight and length on age of the humerus, femur and body of the male and female albino rats.

and millimeters per day) is greater in the former than in the latter.

Chart 1 depicts the values of the growth capacity (grams per 100 grams and millimeters per 100 millimeters per day) of the bones and body weight and length at the stated ages.

It shows that the growth capacity of the femur in weight and length is generally greater than that of the humerus in both sexes up to 65 days of age, and that thereafter the differences are inconsiderable. As a result of this superiority the femur becomes increasingly larger and heavier than the humerus up to the age of 65 days, at which time systemic divagation is brought practically to an end by the approximation to a common level of the growth capacities of the two bones. At 65 days of age, then, the systemic difference has reached the level characteristic of the adult animal. The cessation of deviation at this time indicates that the period between 23 and 65 days of age is the period of systemic development of these bones just as it is the period of their differential chemical development, and that the culmination of puberty at 65 days acts as an equilibrating agent with the one as with the other.

In both sexes the growth capacity of the humerus in length is quantitatively more nearly like that of the femur than is the growth capacity in weight. As a result the increase in difference in length between the two bones is less in degree than the increase in weight difference. Hence the systemic difference in serially homologous bones in the adult as in the immature animal is less a difference in length than a difference in weight.

The ratio of the growth capacity of the humerus to that of the femur in both weight and length is generally of the same order of magnitude in both sexes at all ages. But the chart shows that there are sex differences in the course of the changes in growth capacity with age during the period of differential development. However, the course tends to be systemically characteristic even though sex-specific in type. From this it can be concluded that these systemic relations are practically sex-indifferent. They are however, probably species specific.

It is clear from the chart that there occurs a decrease in growth capacity in weight and length of bones of both sexes on age.

This decrease is not uniform but largely takes place in two stages at which the reduction in growth capacity is markedly abrupt. These occur at 30 and at 65 days of age. The latter brings the growth capacity to the level which is approximately the same for all bones and for both phases of growth, which is the level maintained thereafter, and which is the level characteristic of adult growth.

The reduction at 30 days is attributable to the adjustment to weaning, that at 65 days to the adjustment to puberty. These phases of development have been discussed elsewhere ('25*a*).

There is no apparent systemic difference in degree of response to weaning. With respect to the pubertal reduction, however, a consistent difference is exhibited, in that the degree of cut in growth capacity is greater for the femur than for the humerus in both sexes.

This systemic difference can be considered as a particular expression of the general tendency of the growing organism to reach that state of uniform level of growth capacity of all of its parts, wherefrom those bodily proportions are maintained which characterize the adult of the species. (The basis of this idea has been sketched in an earlier publication ('23*a*): the full discussion will be developed in a later analysis.) Assuming the correctness of the hypothesis, it is obvious that since the growth capacity of the femur during the preceding period of active differential development is greater than that of the humerus, adequate systemic approximation in growth capacity, which is brought about in the case of these two serially homologous components of the osseous system by the culmination of puberty, is only arrived at through a differential systemic reduction in growth capacity such as is shown here.

The chart shows that in both sexes the growth capacity of the femur in weight and length is more nearly like that of the body in degree than is that of the humerus. In other words the growth of the femur follows that of the body more closely than does the growth of the humerus. The precise significance of this systemic difference is obscure. It may be an expression of a response to a need for supporting the greater part of the body weight included in the posterior abdomen. In this connection it

might be noted that the growth capacity of the femur in length generally tends to be slightly greater than that of the body, while that of the humerus is less.

Attention should be directed to the fact that in both sexes there occurs at 65 days of age an approximation of the growth capacity values in body weight and length to a common level, which is quite the same in character as that exhibited by the bones and their constituents, and which is similarly interpretable.

The foregoing analysis demonstrates that the systemic factors participating in the determination of bone growth are largely productive of differences in the degree rather than in the course of development.

Turning now to the inter-sex comparison it is seen from Table 1 that both bones are consistently slightly heavier and longer in the male than in the female save at 23 days of age. The direction of sex difference in the bones, however, is at all ages positively correlated with the direction of sex difference in body size.

A study of Chart 1 reveals the fact there is no consistent direction of sex difference in growth capacity of the bones on age. At 30 and at 75 days of age the growth capacity values are greater in the female than in the male; at 23, 50, and 100 days the reverse is the case, while at 65 days the differences are inconsiderable. It is to be noted, however, that the differences in growth capacity values induced by sex-specific factors affect the bone as a whole and not differentially, in so far as the direction of difference is concerned.

Notwithstanding the inconsistency of sex difference on age, it is a fact that the sex difference in growth capacity in length of both bones is generally less than the sex difference in weight. As a result the degree of sex difference of the bones in length is less than that in weight at all ages.

The general principle is derived from this and the similar relation exhibited in systemic development, that the differentiation of the long bones during growth is due more to the process of growth by increments in weight than to those concerned in growth by increments in length.

This is substantiated by the fact that the increase in sex difference which occurs in both bones, is greater in the weight than in the length relation.

In the analysis of the systemic determinant of bone growth it was shown that a marked reduction in growth capacity takes place at 30 and 65 days of age, and that there is a systemic difference in the degree of reduction at puberty, but not at weaning. It is otherwise in the sex relations, for at both stages of development a sex-difference in degree of reduction in growth capacity obtains. This reduction is uniformly greater in the bones of the male.

The sex differences in the degree of pubertal response are interpretable on the same basis as that given for the systemic differences. The interpretation of the sex-difference in degree of response to the weaning adjustment is, however, another matter. It is probable that a sex-specific factor for ossification is the major influence at this period. This conclusion is based on the fact that an acceleration of ossification or growth capacity for ash, occurs in both bones in the female at 30 days of age, while in the male the usual reduction is exhibited. Since the degree of reduction in the growth capacity of the bone as a whole is dependent upon the degree of reduction of its constituents, it is obvious that a change such as described must produce the numerical dissimilarity exhibited, other relations being approximately the same.

A sex difference in type as well as in degree of development is exhibited in these bones. From the chart it is seen that an acceleration of growth capacity in weight and length occurs in the male but not in the female at 50 days of age. This indicates that a stimulus is active at this time in the male which is not expressed in the female. It is largely a stimulation of the processes of ossification which is sex-specific in character. A like tendency is exhibited in the bones of the female at 75 days of age. The implications of these differences have been discussed elsewhere ('25*a*). All that is necessary to point out here is that the sex factors participating in the determination of bone growth are productive of differences in the course as well as in the degree of differential development, and are thus unlike the systemic factors which apparently are solely productive of differences in degree or rate of growth.

An inspection of the chart shows the presence of a tendency

for the growth capacity of the bones of the male in weight and length to be more typical of that of the body than is that of the bones of the female during the period of active differential development.

The course of development of the bones in weight is sex-specifically different from that of the body. This major difference occurs at the same time in each sex (*e.g.*, during the growth period from 30 to 50 days of age). In the male the growth capacity of the bones increases, while that of the body remains practically constant: in the female the growth capacity of the bones remains practically constant, while that of the body is markedly decreased. , An explanation of these differences is not at hand. It is sufficient for the present purpose to point out that the determination of the course of growth of these bones in relation to that of the body is factored by sex.

From the foregoing it is evident that systemic correlation is closer than sex correlation, in both rate and type of development, particularly during the period of active differentiation. This indicates that the sex-specific factors of influence are superimposed upon the systemic specific determinants of bone growth. These sex-specific factors are probably as much if not more attributable to immediate incretory influences emanating from the gonads, than to the more remote and generalized determinants of genetic origin. This belief is sustained by the fact that the occurrence of these differences is correlatable in time of appearance with the exhibition of certain sex-specific stages in gonadal development. This has been discussed in detail in the report on the chemical differentiation of the bones ('25*a*).

Certain other derivatives are of biological interest.

As a result of sex differences in the relative rates of growth, the length of the bones tends to be greater in proportion to the body length in the female than in the male, at all ages. This indicates that the appendicular skeleton of the female when compared with the axial is longer than that of the male.

A similar superiority of the humerus and femur of the female in weight with respect to body weight is exhibited from 65 days of age on. It is possible that the skeletal system of the sexually mature albino rat is a greater proportion of the body as a whole in the female than in the male.

The weight of the femur per unit bone length is greater than that of the humerus at all ages. The weight of both bones per unit length is greater in the male than in the female in all cases save the femur at 23 days of age. The systemic difference is consistently greater than the sex difference. These differences are structural differences, and indicate, as would be expected, that there is a wider divergency in the nature of the genetic factors concerned in structural differentiation of serially homologous bones, than there is between the sex-differential factors concerned in the development of isotropic bones.

In the comparison of bone growth in length with bone growth in weight ('25*d*) it was shown that there is a high degree of positive linear correlation in both weight and length in both sexes, with body weight and length respectively, and that systemic correlation in both length and weight was also positive, linear and high. The values recorded represented the interstructural association values with the age factor unstabilized.

TABLE II.

FIRST ORDER CORRELATION COEFFICIENTS.

	Male.	*Female.*
Humerus weight and femur weight (body weight constant).....	0.844 ± 0.0042	0.727 ± 0.0030
Humerus length and femur length (body length constant).....	0.647 ± 0.0012	0.402 ± 0.0016

When the general factors for size are eliminated by stabilizing ('26*d*) for body weight in the case of the systemic weight relations, and for body length in the case of the systemic length relations, by the method for partial correlation, the values in Table II. are obtained.

It is evident that in both sexes the systemic correlation in weight is greater than the systemic correlation in length. That is to say the reduction from the zero order value is greater in the length than in the weight relations. This signifies that in the growing animal systemic association in length is more dependent on the general size factors for length, than is the systemic association for weight dependent on the general size factors for weight. This is consistent with the fact that the growth capacity of the bones in weight differs more in type and degree from the

growth capacity of the body in weight, than does the growth capacity of the bones in length differ from that of the body in length. It falls in line with the interpretation that growth of the bones in length is less systemically characteristic than growth in weight; and that growth in bone length is more closely allied to body growth in length, than bone growth in weight is allied to body growth in weight.

The table also shows that systemic association in both weight and length is greater in the male than in the female. This indicates that in the growing animal systemic association is more dependent on general size factors in the female than in the male. The relation is consistent with the fact shown on the chart that the degree of difference between the growth capacity of both bones in weight and length, and that of the body, is greater in the male than in the female.

Summary and Conclusions.

An evaluation of systemic and sex factors as determinants of the type and rate of growth of the humerus and femur of the albino rat has been made.

The results of the analysis indicate that the influence of the systemic factors is largely expressed as differences in degree, while that of the sex factors is exhibited as differences in the course as well as in the rate of growth.

From the point of view of differential development, systemic correlation is closer than sex correlation, both in rate and direction of change, particularly during the period of active differentiation (23 to 65 days of age). From this it is concluded that the sex factors of influence, which apparently are gonadal in immediate origin, are superimposed upon the systemic determinants and direct the sex-specific course of development.

References.

Donaldson, H. H., and Conrow, S. B.

'19 Quantitative Studies on the Growth of the Skeleton of the Albino Rat. Am. Journ. Anat., Vol. *26*, pp. 237–314.

Greenman, M. J., and Duhring, F. L.

'23 Breeding and Care of the Albino Rat for Research Purposes. Wistar Institute Press, Phila.

Hammett, F. S.

'23*a* Studies of the Thyroid Apparatus. XVIII. The Differential Development of the Albino Rat from 100 to 150 Days of Age and the Influence of Thyro-parathyroidectomy and Parathyroidectomy Thereon. Am. Journ. of Physiol., Vol. *67*, pp. 29–47.

'25*a* A Biochemical Study of Bone Growth. I. Changes in the Ash, Organic Matter and Water of Bone during Growth (*Mus norwegius albinus*). Journ. Biol. Chem., Vol. 64, pp. 409–428.

'25*b* A Biochemical Study of Bone Growth. II. Changes in the Calcium, Magnesium and Phosphorus of the Bones during Growth. Journ. Biol. Chem., Vol. 64, pp. 685–692.

'25*c* A Biochemical Study of Bone Growth. III. Changes in the Composition of the Ash. Journ. Biol. Chem., Vol. 64, pp. 693–696.

'25*d* A Comparison of the Bone Growth in Length with the Bone Growth in Weight. Journ. Gen. Physiol., Vol. 9, pp. 63–72.

'26*a* A Biometrical Study of the Size Interrelationships of the Glands of internal Secretion. Journ. Metabolic Research, in press. Cf. Endocrinology, Vol. 9, pp. 297–300.

Jackson, C. M., and Lowery, L. G.

'12 On the Relative Growth of the Component Parts (Head, Trunk and Extremities) and Systems (Skin, Skeleton, Musculature and Viscera) of the Albino Rat. Anat. Record, Vol. *6*, pp. 449–474.